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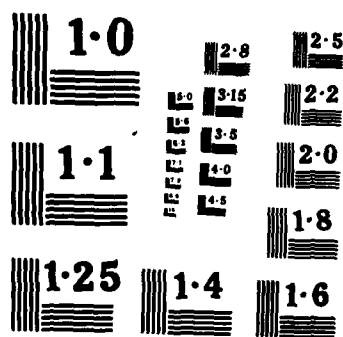
RESEARCH AND DEVELOPMENT OF WOUND DRESSING IN
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RESEARCH AND DEVELOPMENT OF WOUND
DRESSING IN MAXILLOFACIAL TRAUMA
ANNUAL AND FINAL REPORT

Michael H. Gav, Ph.D.

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Report No. 2110-12

RESEARCH AND DEVELOPMENT OF WOUND
DRESSING IN MAXILLOFACIAL TRAUMA

ANNUAL AND FINAL REPORT

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Contract No. DAMD17-81-C-1204

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) <p>Three basic formulations, non-woven fabrics, powders, and microcapsules, of local anesthetic agents, antiseptics, and antibiotics were prepared with poly-L(-)lactide as the polymer. These formulations were characterized by scanning electron microscopy, EDAX (energy dispersive analysis of X-rays), <u>in vitro</u> drug release rate, <u>in vivo</u> efficacy, and stability during storage. The rate of drug release from the <u>matrix</u> was altered by changing drug-polymer ratios, by adding detergent (S.D.S.), by size selection, and by combining fabrics with powders and microcapsules.</p>		

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I. SUMMARY

During the contract period three batches of poly-L(-)lactide were prepared and blended with material in stock to give 1,904 grams of polymer with an R.S.V. of 1.14 dl/g. About 1,000 grams remain. Polymer was used to prepare three basic formulations, non-woven fabrics, powders, and microcapsules, of local anesthetic agents, antiseptics, and antibiotics. These formulations were characterized by scanning electron microscopy, EDAX (energy dispersive analysis of X-rays), in vitro drug release rate, and in vivo efficacy.

Anesthetic fabrics were prepared with benzocaine, bupivacaine-HCl, etidocaine-HCl, and lidocaine base, hydrochloride, and hydrosulfate. Generally the base form of the anesthetics delivers drug in a more continuous manner than the salt forms, and etidocaine-HCl release is more continuous than bupivacaine-HCl release. In these in vitro tests, about 10% of the drug is released in 1 hour and 25% is released within 6 hours. About 60% of the drug remains in the fabric after 24 hours of release testing. These conclusions are based on 20% lidocaine (base), 20% benzocaine, and 15% etidocaine-HCl fabric samples.

Anesthetic powders were prepared with similar materials. Larger particles show more sustained release. Powders release drug more rapidly than the corresponding fabrics.

Sample storage studies indicate that benzocaine samples cannot be stored for one year at 40°C, unless the sample is hermetically sealed. All other tested samples were stable.

Biological testing included Avitene testing by controlled bleeding of a dermatome-inflicted wound, and hemoglobin analysis of the dressing. Efficacy evaluation of sustained-release anesthetic fabrics was conducted. Using a rat sciatic nerve block model etidocaine fabric was shown to induce good local anesthesia.

Four antiseptics, benzalkonium chloride, povidone iodine (PVP-I₂), nitrofurazone, and chlorhexidine diphosphanilate were studied. Benzalkonium chloride at a 20% concentration in fabric gave the best in vitro release (20% immediate release, 35% in one day and 45% in two days).

Povidone-iodine (PVP-I₂) fabrics were prepared from 20% to 75% drug. With less drug the fabric was more fibrous, but drug release was less. A 40% drug fabric was chosen for further testing. EDAX of these fabrics showed less iodine on the surface than the total fabric. PVP-I₂ powders released active iodine rapidly from a 20% drug composite. Povidone iodine microcapsules released drug in a sustained manner for at least 24 hours either as free microcapsules or imbedded in Tegaderm™ or Op-site™. The stability of the drug was studied to provide a guide for preparing efficacious dressings. While the material is stable in vitro significant degradation occurred in serum. In none of the in vivo efficacy studies did povidone iodine formulations successfully control the induced infection, although it was bacteriostatic in vitro.

Nitrofurazone fabrics demonstrated sustained release in vitro which could be altered by changing drug-polymer ratio, adding detergent, and by size selection. While nitrofurazone was shown to be bacteriostatic in vitro, neither pure drug nor powder was efficacious in vivo.

Chlorhexidine diphosphanilate was released in a sustained manner by fabrics, powders, and microcapsules. Since a suitable microorganism was not available at U.S. Army Institute of Dental Research (USAIDR) in vivo efficacy tests were not conducted. Of the three antiseptics, povidone iodine and nitrofurazone do not appear to have the required efficacy. This should be confirmed and chlorhexidine diphosphanilate must still be evaluated.

Two antibiotics, ampicillin and clindamycin, have been utilized. Fabrics prepared from ampicillin (trihydrate and anhydrous) provided slow drug release in all test systems. Release rate could be increased by adding sodium dodecylsulfate (S.D.S.), which is both a detergent and increases the solubility of ampicillin, to the diffusion media or incorporating it into the matrix. Fabric prepared from the more soluble sodium salt released drug very rapidly as did all ampicillin powders. Increasing the concentration of ampicillin in the fabric matrix did not significantly alter the time course of release. Ampicillin microcapsules showed nearly zero order release characteristics. The incorporation of ampicillin trihydrate microcapsules or powders into an ampicillin fabric provided a material with intermediate release characteristics. Ampicillin degradation was a major problem, particularly since the products absorb in the u.v. region and interfered with the drug assay. An H.P.L.C. assay was developed and demonstrated that at room temperature ampicillin was most stable in 40 mM phosphate buffer at pH 6.5. In the in vivo wound model pure ampicillin, 20 mg, did reduce bacterial counts significantly but did not produce a sterile field. Ampicillin fabric (20% mg, of drug) with and without S.D.S. gave a sterile wound in 4 of 5 animals with 6 days of treatment. With higher drug loading (40% and 60%) the fabrics were less efficacious. This is particularly confusing since the in vitro release characteristics of all ampicillin fabrics were similar and significant amounts of ampicillin were present in both the wound and dressing at the end of the treatment period. However, only in the first day was drug occasionally detected in guinea pig serum.

Clindamycin fabrics, powders, and microcapsules have been prepared and tested. The fabric released drug rapidly in all test systems and release was not reproducible. Similarly, poor results were obtained in the in vitro studies of clindamycin hydrochloride powders. The initial microcapsule preparation also showed very rapid release, which is attributed to the low coating level. In vivo efficacy tests were not conducted with clindamycin formulations since a suitable organism was not available at USAIDR.

FOREWORD

In conducting the research described in this report, the investigator(s) adhere to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of (DHEW Publication No. (NIH) 78-23, Revised 1978).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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POLYLACTIDE POLYMERS

Viscosity vs. Molecular Weight

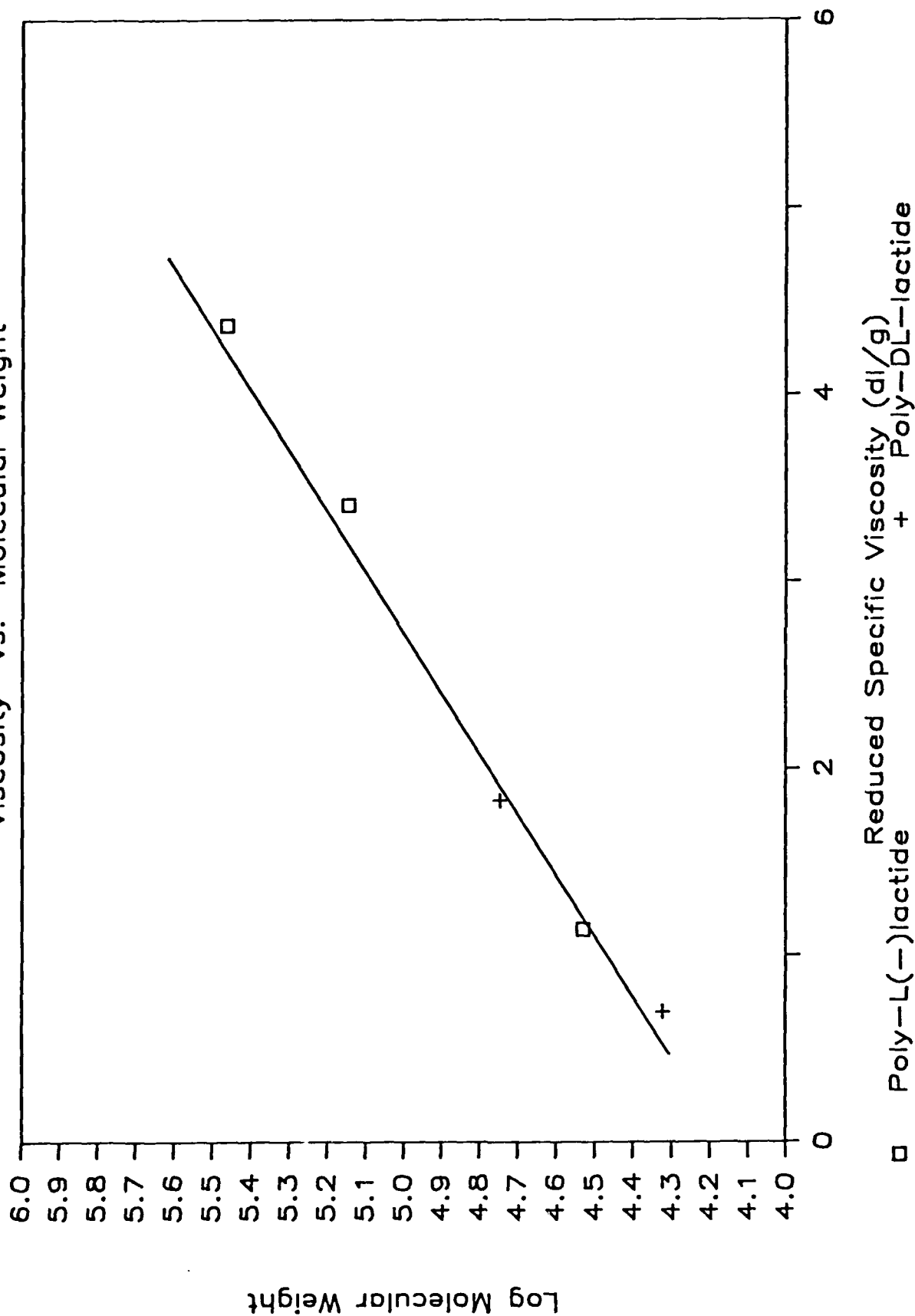


TABLE 2
POLYMER MOLECULAR WEIGHT DISTRIBUTION

Sample: Poly-(L-)lactide (R.S.V. = 1.19 dl/g)

Number Ave. MW (MW_n)	9,440
Weight Ave. MW (MW_n)	20,100
Polydispersity (MW_w/MW_n)	2.14

All six batches of polymer were blended together after dissolving in methylene chloride. A total of 19.3 liters of this polymer solution was precipitated by slow addition of isopropyl alcohol in the ratio of 3 to 1 (57.9 liters IPA). Due to the large volumes this had to be carried out in three batches.

After precipitation, the polymer was removed from the solution by vacuum filtration, the pieces were pressed with rubber dam material in the Buchner funnel to remove most of the solvent. The polymer was then placed under vacuum to remove residual solvent. Due to the low volatility of isopropanol and the large volume of polymer, the drying process took two weeks. When the polymer chunks were sufficiently dry, they were ground in a blender to a uniform size, shaken in a large bag and again placed under vacuum to complete the drying. Three random samples of this final mixture were taken and their viscosities determined in duplicate. The viscosity was found to be 1.19 ± 0.03 dl/g and the total polymer obtained was 3,048 grams which was a 90% yield.

The polymer was stored under argon in the freezer. The polymer was placed in plastic bags into one-gallon cans which are tightly sealed (paint cans). The air was removed by vacuum and replaced by argon, just prior to sealing. This procedure has been standardized, but is presumably much more stringent than necessary to prevent polymer degradation.

2. Polymer Characterization

A sample of polymer was sent to Cambridge Analytical Associates for determination of molecular weight distribution by gel permeation chromatography. Samples were dissolved in hot trichlorobenzene. Duplicate injections of the samples were run 12 hours apart to determine whether the high temperature analysis would degrade the polymer. After 12 hours, no evidence of polymer degradation was evident. A series of Micro-Styrigel columns (10^3 , 10^4 , 10^5 , 10^6 Å) from Waters Associates were used at 145°C . The flow rate was 1.5 ml/min. and the change of refractive index was used for detection. The molecular weight determination was made by reference to polystyrene standards.

The data of molecular weight distribution was calculated by Cambridge Analytical Associates, based on the polystyrene standards curve for the gel permeation chromatography (GPC) columns. A more appropriate calculation is based on the relative size of the polylactide and polystyrene molecules. The standard value for polystyrene is 41 atomic weight units per angstrom. For polylactide, a calculation using standard bond lengths and angles yields 18 atomic weight units per angstrom. The molecular weight data based on this calculation is given in Table 2. This weight-average molecular weight (M_w) is in agreement with the data of Nuwayser, et al (1976), see also Figure 1.

Further analysis of the GPC peak indicates that the actual molecular weight distribution is quite broad. The raw data of refractive index of the collection tubes was analysed as a Gaussian curve for a similar polymer. The mean M_w was 34,400 (polydispersity 2.33). At ± 1 S.D. from this mean, the molecular weights are 7,200 and 50,400. This analysis

TABLE 1

POLYMERS PREPARED FOR COMBINED PROGRAM

<u>Polymer No.</u>	<u>Starting Grams Dimer</u>	<u>Approximate R.S.V.</u>
5-6-6	574	1.25 [✓]
5-6-10	649	1.02 [✓]
5-6-15	582	0.57
5-6-18	600	2.91
5-6-19	400	> 3.0
5-6-21	665	1.08 [✓]
5-6-23	617	> 3.0
5-6-25	637	2.23
5-6-27	500	0.72
5-6-29	500	2.08 ⁺
5-6-30	500	1.57 [✓]
5-6-31	300	0.92
7-12-2	500	1.40 [✓]
<u>7-12-7</u>	<u>500</u>	1.29 [✓]
TOTAL	7,524	

[✓] used in blend (total of 3,388 gm)

⁺ exchanged with NIH program for last two polymers on list

III. ACCOMPLISHMENTS

A. Polymer Synthesis and Characterization

1. First Polymer Batch Preparation

Poly-L(-)lactide of a reduced viscosity of about 1.0 dl/g has been prepared and used by us for several government contracts. This was the polymer used for the preparation of anesthetic microcapsules by the Wurster process under Contract Nos. DAMD17-79-C-9019 and DAMD17-81-C-1195, and for powder and fabric formulations of various anesthetics and antiseptics under Contract No. DAMD17-80-C-0093. It was also the polymer used for much of the steroid encapsulation work performed under Contract No. N01-HD-3-2738. The same method of preparation was used in the present contract (DAMD17-81-C-1204).

The preparation of the polylactide was performed as follows: The lactide dimer was obtained from Boehringer-Ingelheim through Henley and Company (N.Y.C., N.Y.). This dimer was purified by repeated recrystallizations from ethyl acetate shortly before use. Reagent Grade solvents were used for all operations. The lactide dimer was heated in a 120°C oil bath while stirring the melt, in vacuum, for 30 minutes to remove traces of volatile materials. An inert gas was then introduced to release the vacuum. Next, the bath temperature was raised to 180°C. To this mixture was added 0.2 ml of stannous octoate catalyst. Within about 15 minutes after the catalyst addition, the polymer mixture reaches a maximum temperature. After about 5 hours, the reaction is stopped by removing the mixture from the oil bath, and the polymer is allowed to cool to room temperature. The polymer block is dissolved in methylene chloride and the solution is decanted and treated with three volumes of isopropanol, by slow addition to a stirred solution.

Two kilograms of poly-L(-)lactide which has a reduced specific viscosity (R.S.V.) between 1.0 and 1.5 dl/g were required for this contract. A parallel contract (DAMD17-81-C-1195) also required an equivalent amount of polymer of the same R.S.V. To improve the characterization and reproducibility of both programs, it was decided to combine the requirements of both programs in one blend of a number of batches of polymer.

Approximately 1,200 grams of poly-L(-)lactide (R.S.V.=1.0 to 1.2 dl/g) was available from a previous contract (DAMD17-79-C-9020). This material was used for preliminary experiments on both contracts to quantify operating parameters.

Because of the narrow range of the specified R.S.V. and the excessive heat generated in large batches, preparation of this quantity of polymer was a lengthy process. A total of 12 batches (see Table 1) of dimer were polymerized for these two contracts. Four of these were in the right range and eight were not. Five of the eight batches were too high and three were too low. Two of the out-of-range batches were exchanged with a similar project which had two batches which fell within our range but which were out of range for that application.

The fabric form of a wound dressing offers obvious advantages over a solution or a powder. However the fabric may require a backing as discussed in the earlier section. This backing is presently a gauze bandage material and it serves as a target for spraying and as a form for cutting the fabric to known size. Without backing the material can be readily compressed or stretched. There may be significant advantages to materials such as Avitene mat, or wound dressings such as Op-site™ or Tegaderm™ as the backing material.

For promotion of wound healing some of the materials which are being intensively studied as burn dressings may offer special advantages. USAIDR (Col. Vincent) observations that the wound dries out excessively with time, suggests that a backing should be used which prevents excessive water loss. Suggested materials include Op-site™ (Smith and Nephew, Acme United, Fairfield, Conn.) and Tegaderm™ (3M, St. Paul, Minn.). Op-site™ and Tegaderm™ are thin polyurethane films with good water vapor and oxygen transmission properties. They are supplied with a contact adhesive of polyvinylether or acrylates.

Powders are more difficult to apply uniformly and reproducibly to an open wound. The powder can be poured into the wound, sprayed from a powder sprayer such as an insufflator, or applied to a wound dressing prior to application to the wound. All three approaches have been considered.

The povidone iodine microcapsules have been sent to USAIDR on Tegaderm™ and in unit dose form in heat-sealed packets. Commercially available blister packs are available which allow delivery to a specific location. An individualized container having a breakable spout on the blister pack is possible (Morton Salt, discontinued).

Application to the bandage is a common method of medicating a wound. Using the acrylate contact adhesive of dressings such as Tegaderm™, a layer of powder could be uniformly applied to the wound. This material is available from 3M as Tegaderm™ and as precoated transfer adhesive systems. Multiple layers of microcapsules should be attachable using adhesive approaches. Very thick layers of microcapsules would be more readily achieved by filling a spacer grid placed between two layers of gauze which would act as a sieve to prevent the loss of drug particles. This is a practical problem which can be solved at a later time.

some data on the effectiveness of phosphanilic acid against *Staphylococcus aureus*. Lee, et al (1980) describe the bioavailability and metabolism of phosphanilic acid. In general the activity is similar to the sulfanilamides.

c. Anesthetics

Several anesthetic drugs have been tested on this contract. These have included benzocaine and the amide anesthetics, lidocaine, bupivacaine and etidocaine. The amide anesthetics have been tested in the free base form and as the hydrochlorides. Lidocaine has also been prepared as the sulfate and phosphate salts. Benzocaine is a weak anesthetic, but one which has high lipid solubility and is effective transdermally. Lidocaine is the anesthetic of choice for many local anesthetic procedures. Bupivacaine and etidocaine are more potent anesthetics, but have not had the extensive history of usage of lidocaine. In similar polymer-drug matrices, the rate of drug release parallels the drug solubility. This is true of microcapsules, non-woven fabric, and comminuted powders. Drug solubilities have been measured by ultraviolet absorbance of saturated solutions. The values are:

Lidocaine-HCl in water	570 mg/ml
Lidocaine-base in water	3.5
Lidocaine-HCl in phosphate buffer	240
Etidocaine-HCl in phosphate buffer	24
Bupivacaine-HCl in phosphate buffer	39

The testing of these anesthetics was performed in conjunction with the parallel contract on microencapsulated anesthetic (Contract DAMD17-81-C-1195), and we were guided by the results of the animal studies on that contract.

d. Hemostatic Agents

The non-woven fabric wound dressings absorb a considerable volume of fluid from a wound site. However, the matrix material is polylactide, which is not expected to promote clot formation. The material is used because it is well tolerated as a body implant and is biodegradable to lactic acid. The non-woven fabric is normally sprayed on a gauze bandage material. However other backing materials have been tested. Avitene™ (Avicon Corp., Fort Worth, Texas) is an excellent hemostatic material and it has been incorporated into a useful non-woven fabric form by FMC Chemicals Division, Princeton, New Jersey. This material has been supplied to us by Dr. Strange and has been used as an alternative backing (target) for the BIOTEK non-woven fabric wound dressing. In earlier studies we used the bulk form of Avitene™ and Gelfoam™. These materials were more difficult to incorporate into a wound dressing.

3. Wound Dressing Application Systems

The convenience of application of the wound dressing is important since the dressing should be applied very rapidly after the wound is cleaned. Also a convenient package allows a known dose to be applied to a specific wound area.

Norwich-Eaton Pharmaceuticals sells nitrofurazone as Furacin™ soluble dressings, cream, powder and solutions. For product support they list 56 major human clinical studies. Early work on wound treatment was performed by the military (Snyder, et al, 1945, McCollough, 1947).

Cedergren, et al, (1952) wrote an extensive article and noted the importance of the correct ointment base. Friedgood and Ripstein (1953) noted that sensitive wounds were more easily treated by sprinkling of a powder than by applying ointment or changing dressings. They preferred a powder insufflator to a shaker-top vial.

Zydek (1954) treated 630 wounds, including 287 gash wounds. Results for the gash wounds were listed as very good (148), good (139), questionable (0), and unsuccessful (0).

Gilliotte (1960) also tested a shaker-top vial and preferred a squeeze bottle having a nozzle orifice (insufflator). He used the powder on 256 patients, including 155 traumatic lacerations and 3 other avulsive injuries.

Frenmil and Fishel (1970) compared several nitrofurazone dressing methods in 195 patients with various wounds. Healing was rapid or normal in 93% of the cases. Sensitization occurred with 4 patients and slow healing occurred in 9 cases (13-33 days).

Cedergren, et al (1952) also discussed hypersensitivity. Sensitivity is a problem with long term use of the drug and is exacerbated by use of certain vehicles. It is not considered a serious problem by any of these investigators when nitrofurazone is used for traumatic injuries.

Chemically, nitrofurazone is quite insoluble in water. The drug is relatively stable, although sunlight and excessive heat should be avoided. It is less stable than BAC but is more stable than povidone iodine.

Most recently chlorhexidine diphosphanilate was added as a potent antiseptic drug. This is a proprietary product which is available only from Westwood Pharmaceuticals (Bristol Laboratories). It had been made available to the U.S. Army Institute of Surgical Research, and tested by Dr. McManus. We received 100 grams of this material from Westwood Pharmaceuticals for our in vitro and in vivo studies.

Remington's Pharmaceutical Sciences (16th Ed. 1980) states that chlorhexidine is bactericidal to both gram-positive and gram-negative bacteria, although it is not as potent against the latter. In a 4% aqueous solution as a surgical scrub, it decreases the cutaneous bacterial population more than either hexachlorophene or povidone iodine. Chlorhexidine is used for the preoperative preparation of both surgeon and patient for the treatment of burns and the irrigation of wounds and surgical infections. Chlorhexidine was obtained as the diacetate from Sigma Chemical Company.

Phosphanilic acid (p-aminobenzenephosphonic acid) is not readily available, nor is there extensive literature on this compound. Thayer, Magnuson and Gravatt (1953) describe the antibacterial action of several compounds, including phosphanilic acid. Kanitkar and Bhide (1947) give

microbiocidal spectrum. It is lethal to both Gram-positive and Gram-negative bacteria, as well as many yeasts, fungi, protozoa and viruses. It is a widely used antiseptic, especially under the tradename, Betadine™. A relatively large quantity of povidone iodine is required to maintain antiseptic conditions.

Later in the program a quaternary ammonium surfactant, benzalkonium chloride (BAC) was added as an alternative antiseptic. It is used at low concentration levels and is a very stable material.

Potential suppliers of benzalkonium chloride preparations were contacted. Discussions with Sterling-Winthrop personnel indicated that the alkyl group should be approximately n-C₁₂. However, no unformulated benzalkonium chloride was available. Mason Chemical Company was suggested as a primary supplier of quaternary ammonium complexes. Information was obtained on various commercially available materials and the fabric (e.g., diaper) bacteriostatic formulations. Mason Chemical Company supplied three standard formulations which were 80% solids in an isopropanol-water solvent. The solids are:

<u>Product</u>	<u>Percent of</u>			
	<u>C₁₂</u>	<u>C₁₄</u>	<u>C₁₆</u>	<u>C₁₈</u>
Maquat LC-12S	61	23	11	5
Maquat MC-1416	5	60	30	5
Maquat MC-1412	40	50	10	-

LC-12S is the standard benzalkonium chloride of commercial interest. The solvent was evaporated from the three Maquat samples.

Small quantities of benzalkonium chloride are required for bacteriostatic efficacy. Zephiran is sold as a 1/750 dilution (1,333 ppm). This concentration is required for Pseudomonas bacteriocidal activity. For diapers about 400 ppm is used, which corresponds to approximately 20 ug/square centimeter.

Nitrofurazone was later added as an alternate antiseptic. Nitrofurazone (Furacin™, Norwich-Eaton) is used as a local antibacterial agent. It has a wide spectrum of activity and most bacteria of surface infections of the skin or mucosal surfaces are sensitive to the drug. Bacteria develop only a limited resistance to the drug and cross-resistance with sulfonamides and antibiotics does not occur. Nitrofurazone retains its activity in blood, serum and pus. Phagocytosis is not inhibited and it does not interfere with wound healing (Remington's Pharmaceutical Sciences, 14th Ed., 1184). For the best effect it should be applied for at least 24 hours; hence a drug release formulation would be advantageous. Nitrofurazone concentrations for bacteriocidal action are approximately 1:100,000. Hence small quantities would be required in a wound dressing. In reading secondary sources such as Goodman and Gilman's "The Pharmacological Basis of Therapeutics" (6th Edition, 978-9), nitrofurazone is compared very favorably with other antiseptics.

2. Selection of Drugs

a. Antibiotics

The selection of antibiotics was made at USAIDR, with respect to the materials which were being developed by other contractors in their programs on maxillofacial wound dressings. Ampicillin and clindamycin were chosen for this program.

Ampicillin is available from several sources and is available in several physical and chemical forms. Commercially available forms are as the sodium salt (crystal or lyophilized) and as the acid (anhydrous or trihydrate). Bristol Laboratories is the major domestic supplier of ampicillin, and a good source of technical information. Sodium ampicillin and the acid trihydrate were obtained from Bristol Laboratories. A small quantity of anhydrous ampicillin was obtained from Sigma Chemical Company. Lyophilized sodium ampicillin was purchased from H. Reisman Corporation, (Orange, NJ).

Clindamycin is available only from Upjohn and is under patent protection. It is available as the simple hydrochloride or as various esters. For our slow release product we would not require an esterified material. One hundred grams of clindamycin-HCl (Cleocin™ U-21251F Potency 864 ug/mg) was requested and received from the Upjohn Company. The base form of clindamycin is readily prepared by titrating the hydrochloride in water with sodium hydroxide. The crystals are collected on filter paper and washed with small quantities of water.

b. Antiseptics

The use of antiseptics in a wound is of obvious importance. The wound area is localized and the microbial contamination covers the gamut of viable aerobic and anaerobic bacteria, spores, fungi, and yeasts. A high concentration of antiseptic is required for the initial microbial challenge. However, time of contact is also important, and the relative therapeutic effectiveness of intermittent and continuous dosage regimens for antibacterial agents is uncertain (Toothaker, Welling and Craig, 1982). Correlations have been attempted with various pharmacokinetic parameters such as the maximum plasma concentration and the time during which the drug level exceeds the minimum inhibitory concentration. Too high a concentration of an antiseptic may cause tissue cell damage and too short a time period may not allow contact during the life cycle of the bacterial cell at which it is most susceptible to biocidal agents. Thus a time release of antiseptic agents should be useful. Specific studies (e.g. Georgiade and Harris, 1973, for povidone iodine) have shown significant advantages of multiple dosing regimens for antiseptics.

The first antiseptic agent tested in the BIOTEK program was iodine. The elemental form could be incorporated into polylactide matrices, but was lost by iodine sublimation. Povidone was then added as an iodine carrier. In an aqueous system this polyvinylpyrrolidone complex maintains a constant iodine concentration. Povidone iodine possesses a broad

Infection of the wound site is the most common medical complication after the initial blood loss and shock symptoms have been stabilized. Combat wounds are characterized by a high incidence of infection. This may be due to the presence of devitalized tissue, the presence of foreign bodies in the wound, and/or because there is an unavoidable delay in treating wounds in a combat situation. The immediate treatment may require cleansing the wound area, prior to bandaging. The effectiveness of this procedure depends both on the skill of the paramedic and on the field conditions. The infection process is peculiar to the microorganisms causing the infection, and to the mode of operation of the antibiotics or antiseptics which are used to combat the infection. However, the advantage of the continuous presence of the anti-infective agent at the wound site has been demonstrated for many wound dressings, and also for systemic antibiotic (p.o., i.v., etc.) regimens used to control local infections. Finally, the use of biodegradable polymers in this application is advantageous, since fragments of the wound dressing which are left in the wound will be absorbed without incident.

C. Technical Background

1. Previous Contract Experience

Under the predecessor contract (DAMD17-79-C-9020) biodegradable polymers were blended with various drugs which could be used as wound dressings in maxillofacial trauma. Three local anesthetics (procaine, benzocaine, and etidocaine) two antiseptics (iodine and pyridinium chloride), an anti-inflammatory steroidal drug (hydrocortisone) and epinephrine were studied in non-woven fabric, film and powder forms. Drug release was measured as a function of time for 45 different polymer-drug combinations. Gelatin (Gelfoam™) and collagen (Avitene™) were studied as contact hemostatic agents. These materials were embedded into the polymer surface.

Many of the polymer-drug composites which were prepared on this Abcor contract demonstrated a drug release profile which was in the appropriate range for wound dressings. Under this contract both the polylactide and polylactide-co-glycolide polymers were used. For the polylactides both the poly-L(-)lactide and the poly-DL-lactide were investigated, with two molecular weights of the poly-L(-)lactide being used. Based on the results of this contract, we proposed to work only with the higher molecular weight poly-L(-)lactide on the present contract.

For the antiseptic material we substituted the povidone-iodine complex for pure iodine on the present contract. Although it was possible to incorporate considerable iodine into the polymer matrix by codissolving the polymer and iodine in methylene chloride, iodine was lost by sublimation and perhaps by reaction with the polymer. Also, all of the iodine which was released from the drug-polymer matrices was released immediately. Thus, we proposed to use the more stable povidone-iodine complex in future work. The incorporation of antiinflammatory and vasoconstrictive drugs was considered to be potentially contraindicated for a general wound dressing, due to their interference in the normal wound healing process.

II. INTRODUCTION

A. Objective

The initial objective of this contract was to incorporate antiseptic, anesthetic and hemostatic agents into a single wound dressing, with slow release of the antiseptic and anesthetic agents for optimum effectiveness with the fewest changes of the wound dressing. The polymer used in the drug polymer matrix would be biodegradable (poly-L(-)lactide), allowing fragments of the wound dressing to be left in the wound and absorbed without incident. Initially on this contract, homogeneous drug-polymer formulations of non-woven fabric and powders were prepared and tested in vitro. On a sister contract (DAMD-17-80-C-0110) microcapsules (core/wall particles) of local anesthetics were being prepared and tested, in vitro and in vivo.

In a later modification of the contract microencapsulation of antiseptic drugs was added and hemostatic agents were removed from the scope of work. At a later time local anesthetics were removed from this work scope, and the local anesthetic microcapsule contract was terminated. The last contract modification added antibiotic drugs to the work scope of this contract.

B. Military Relevancy

A combat injury to the maxillofacial area requires immediate attention to stop the bleeding and generate a sterile site for wound healing. The injured soldier may also be required to continue fighting or be able to evacuate the area under his own power. Thus a general analgesic agent such as morphine may not be useful, and a local anesthetic agent would be advantageous.

For less severe injuries, sustained-release drug delivery in military medicine would allow personnel to perform vital combat functions after receiving injuries which would otherwise require removal from the battlefield.

Hemostasis is the first task of the soldier or paramedic. Pressure and wound dressing contact are necessary immediately. The more surface area of dressing contact, the faster the bleeding will stop. Certain materials, such as collagenous protein (e.g. Gelfoam[™], Avitene[™]) offer specific interaction with blood components (platelets) and are excellent hemostatic agents. After the initial blood loss, capillary bleeding may continue. This should be minimized, but blood flow to the site of the injury should not be compromised.

Recently, acute pain treatment has been advocated which deliver sufficient analgesic for pain without the patient requesting additional drug. This approach eliminates the learned pain response which so often leads to drug dependence following PRN dosing (U. Washington, School of Medicine, 1983). Pain treatment for soft tissue damage is normally scheduled for 2-3 days. For bone involvement the pain treatment extends for a week or two. Slow release anesthetics would aid in this time-contingent management of acute pain.

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indicates that the broadening of the molecular weight distribution by mixing polymers of different viscosity ($+ 0.5$ dl/g) may be insignificant compared with the distribution within a single batch of polymer.

This broad molecular weight distribution was also found to be natural for the process. The theoretical polydispersity of a completed condensation polymerization reaction is 2.0 (Flory, 1953). The polydispersity (M_w/M_n) of our polymer blend is 2.14.

To prove that batches with widely varying viscosities could be blended, a sample of 0.73 dl/g polymer (5-6-27) and 2.04 dl/g polymer (5-6-25) was analyzed by gel permeation chromatography. Also a combination of 33% of the low and 67% of the high molecular weight polymer was analysed. The results are summarized in Table 3 and compared with the initial polymer analysis of Contract No. DAMD17-80-C-0110. At equal concentrations of the two polymers (0.73 and 2.04 dl/g) the chromatogram overlap is 48% (Figure 2). The combination chromatogram does not show a bimodal distribution but does show a tailing at high molecular weights.

Thus we could blend this wide a viscosity range without affecting the polymer coating and drug release properties of the polymer blend.

For Contract DAMD17-81-C-1204 we indicated that the use of poly-L(-)lactide of R.S.V. = 1.2 dl/g from batches of R.S.V. between 1.0 and 1.5 dl/g was unnecessarily restrictive. Since the polymerization process produces a wide range of molecular weights, a range of 1.2 ± 0.6 was proposed.

3. Second Polymer Batch Preparation

When the supply of polymer which had been prepared for Contracts DAMD17-81-C-1204 and DAMD17-81-C-1195 had been exhausted, a new batch of polymer was prepared for use only on Contract DAMD17-81-C-1204. Lactide dimer from Boehringer-Ingelheim was recrystallized from ethyl acetate. The goal was to produce three batches of 500 grams each, which had reduced specific viscosities (R.S.V.) of 1.2 to 1.8 dl/g. This could be blended with polymer in stock, which had an R.S.V. of 0.70 dl/g, to give a blend having an R.S.V. of 1.2 ± 0.1 dl/g.

For the first polymerization batch, the dimer was recrystallized twice. The polymer product was dissolved in methylene chloride and a small sample was reprecipitated using isopropanol. The R.S.V. of this material was 1.69 dl/g. For the next polymerization the dimer had to be recrystallized three times, and the R.S.V. of the product was 1.53 dl/g. In the final batch the dimer was recrystallized three times and the R.S.V. was 1.76 dl/g.

These three batches had a combined weight of 1506 grams and were blended with 1000 grams of 0.70 dl/g polymer. This material was precipitated with isopropanol and the final blend had an R.S.V. of 1.14 dl/g. The product was dried in the air suspension chamber, and 1904 grams of polymer was recovered. Thus the total yield was 76%. This apparently lower yield is probably due to better drying of the polymer. The previous

TABLE 3
EFFECT OF VISCOSITY (R.S.V.) ON POLYMER BLENDS

<u>R.S.V.</u>	<u>Polymer</u>	<u>MWw</u>	<u>MWn</u>	<u>MWw/MWn</u>
0.73	5-6-27	29,880	17,590	1.70
2.04	5-6-25	87,840	52,020	1.69
1.20*	Blend of Above	57,420	22,860	2.51
1.19	Previous Contract Blend	45,700	21,500	2.14

* calculated R.S.V.

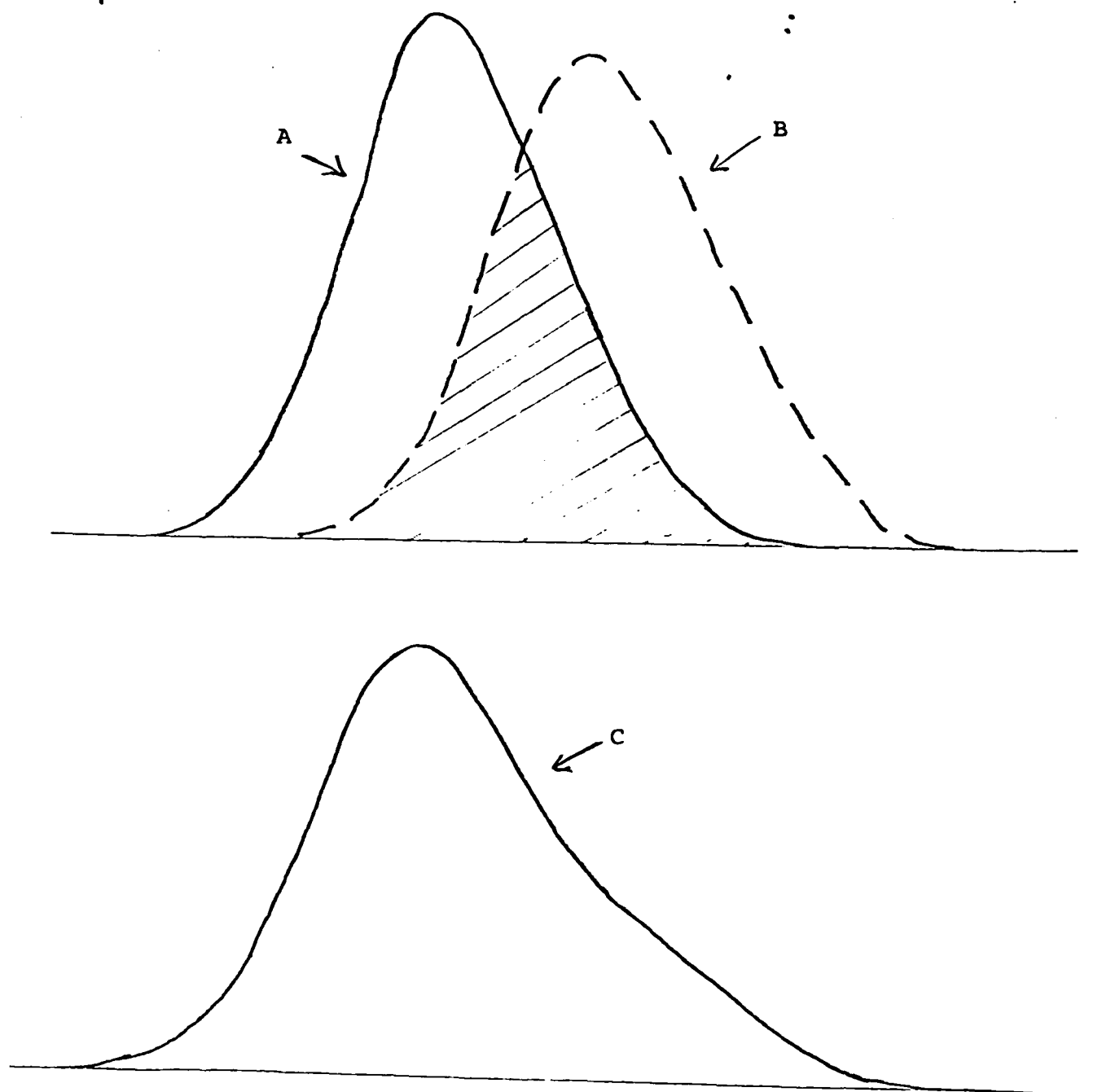


Figure 2 Gel Permeation Chromatograms of
A. 5-6-27, R.S.V. = 0.73 Pd = 1.70
B. 5-6-25, R.S.V. = 2.04 Pd = 1.69
C. 2 of 5-6-27 and 1 of 5-6-25
R.S.V. = 1.2 Pd = 2.51

polymer was not dried in the air suspension chamber, and a later GC analysis of the polymer indicated 15% isopropanol. The present polymer is dry, and has been sealed under argon in metal cans. It was stored in the freezer.

B. Development of Polymer/Drug Materials

1. Non-Woven Fabric Preparation

The polymer-drug fabric is prepared in a manner similar to that of Contract DAMD17-79-C-9010. About a 10% (weight-to-volume) solution of the polymer is prepared using methylene chloride as the solvent. The appropriate amount of drug is added to this system to obtain the appropriate drug/polymer ratio (e.g. 20%). This solution is poured into a tank which is connected to the liquid inlet of an air atomizing nozzle using a piece of tubing.

The air inlet is connected to a source of compressed gas. Fine polymer-drug fibers are formed as the solution leaves the nozzle. The fibers are collected on a piece of surgical gauze. This material is held at a fixed distance from the gun nozzle and is attached to a frame to keep it taut. Thus air passes through the wound dressing to facilitate drying of the solvent.

A series of experiments were performed to determine the spraying characteristics of solutions which yielded the type of non-woven fabric tested during this contract. Initial experiments were performed with polymer only. Later experiments included various concentrations of lidocaine-HCl at a constant polymer concentration in methylene chloride.

In the initial experiments, five grams of polymer were sprayed at the target from various concentrations in methylene chloride. The viscosities of these solutions were measured using a falling ball viscometer. This was correlated with the time required to spray the solution through the atomizing nozzle. The density of the solution was also measured, but it remained constant (1.29 gm/cm^3). A sample of fabric was cut with a punch and weighed and measured. The thickness was approximated using vernier calipers, since the standard Ames thickness gauge severely compressed the sample. A density was calculated from the thickness, area, and weight. The data are shown in Table 4.

A linear relationship was found between the solution viscosity and spraying time:

$$\eta = 16.2t - 16.6$$

where, η is viscosity in centipoise
and t is seconds/milliliter of solution.
The correlation coefficient was $r = 0.9997$.

Less fibrous product was collected in the center of the target when the solution concentration was lower. The density of the fabric may also be higher when the solution concentration is lower. The combination leads to much less visible fabric at lower concentrations.

TABLE 4
SPRAYING OF NON-WOVEN FABRIC OF POLYMER

<u>Solution Concentration (g/100 ml)</u>	<u>Solution Viscosity (cps)</u>	<u>Spray Time (sec/5 gm)</u>	<u>Sample Weight (mg/3 cm²)</u>	<u>Sample Thickness (cm)</u>	<u>Sample Density (mg/cm³)</u>
9	16.1±0.2	112	40	0.28	60
10	22.5±0.0	121	31	0.16	81
11	29.1±0.4	129	56	0.47	50
12	42.0±0.2	150	61	0.41	62
13	(57)*	175	59	0.50	49

* calculated from spray time

At 11% polymer in methylene chloride, various drug/polymer ratios were sprayed. Lidocaine-HCl was sprayed at 5, 10, 20, 40% drug loadings as shown in Table 5. Again the more concentrated solutions gave the greater quantities of collected fabric. However, the solution density, viscosity, and spraying times were all similar. Since the mat thicknesses were about the same, the fabric densities were highest for the fabrics with the most drug (see Table 5).

2. Powder Preparation

The second form of the BIOTEK wound dressings is particulate. In this form combinations of powders can be added as required. Separate powder particles can be hemostatic or contain antiseptic or anesthetic agents. Separate particles can be tailored for optimal delivery of a specific drug. Microencapsulation of povidone iodine has been shown to yield the best continuous in vitro release of active iodine. These oxidizing particles can probably be added to particles of less stable anesthetics such as the base form of lidocaine, provided the system is kept dry. A non-woven fabric containing both of these materials has been demonstrated to be unstable, under ambient room conditions.

Powders have been made by by comminution of a homogeneous film of polylactide and drug. In this approach the drug release is expected to be non-linear. The mathematics generally follow the Higuchi equation ($Q = kt^{1/2}$). The rate of release decreases with time as the surface layers of the particle or fiber become depleted of the drug. Baker and Lonsdale (1974) have published the equations for these systems. Since more drug may be needed initially, this approach of using homogeneous drug/polymer materials is appropriate for wound dressings. However, in many cases, drugs are not released from the center of the particle or fiber within a reasonable time period. This is especially true of high molecular weight materials such as povidone iodine.

In the previous contract work (DAMD17-79-C-9020), powders were prepared by comminution of material prepared as a film, by grinding at liquid nitrogen temperature. Both mortar and pestle and micro-mill apparatus was employed. since these methods cannot be readily scaled up to common pharmaceutical equipment, different methods were employed in this contract work.

Since the previous powders which showed good drug delivery had sizes of 50 to 500 microns, it was important to maintain this approximate size range. Thus micronization in a fluid energy mill would presumably not be advantageous. Felmeister (1970) describes the common apparatus and methods of producing powders for the pharmaceutical industry. Of the intermediate pulverizers described, the hammer mill is the most applicable to the present materials. A cooled mill would be most appropriate for large scale production. For small scale production the sample was sent through a standard mill with dry ice.

Solutions of 20 grams of drug and 80 grams of polymer were prepared using 700 ml of methylene chloride. Films were cast onto 400 square centimeter area of glass plates. These films were cut into squares which were fed with the auger into the hammer mill.

TABLE 5

SPRAYING OF NON-WOVEN FABRIC WITH LIDOCAINE

(11% Polymer in Methylene Chloride)

<u>Lidocaine-HCl</u> <u>% of Polymer</u>	<u>Sample</u> <u>Weight</u> <u>(mg/3 cm²)</u>	<u>Sample</u> <u>Thickness</u> <u>(cm)</u>	<u>Sample</u> <u>Density</u> <u>(mg/cm³)</u>
5	43±10	0.36±0.05	64±4
10	70±18	0.46±0.09	62±5
20	96±25	0.44±0.12	103±23
40	118±22	0.42±0.10	131±20

A Mikro-Pulverizer C.F. (18,000 RPM, Pulverizing Machinery, Div. Slick Industrial Co., Summit, N.J.) was used. The mill was assembled with the hammer edge of the rotor facing the direction of rotation. A 20 mesh (841 μ m) classifier screen was used at the bottom of the mill. The feed and comminution chamber were pre-cooled with 500 grams of dry ice. Next the sample was sent through the mill, mixed 1 to 10 with dry ice (i.e., 90% carbon dioxide). A final charge of 100 grams of dry ice was sent through the mill to increase the yield from the chamber. The mill was dismantled, washed, dried, and reassembled for each drug run.

3. Microcapsule Preparation

The second method of making wound dressing particles is by microencapsulation. In this approach a particle of drug is coated with a layer of polymer. A continuous constant release is possible with this approach, since a constant concentration gradient can be maintained across the wall of the microcapsule. In practice some non-linearity is observed in the rate of release, because the microcapsules are of varying sizes and have imperfections in the capsule walls. However, drug release is more continuous than for homogeneous systems, and can approach a constant rate in some cases (e.g. Nuwayser, *et al*, 1976).

Under Contract DAMD17-81-C-1195, entitled Local Anesthetic Microcapsules, lidocaine, etidocaine and bupivacaine have been microcapsulated with polylactide, using the Wurster process. This work has generated microcapsules having a wide range of *in vitro* release rates. The rates are dependent on the choice of drug, the amount of polymer coating and the size range of the microcapsules. Slow release has been demonstrated *in vivo* by measurement of circulating blood levels of the anesthetics in rabbits. Lower systemic toxicity (4-7 times less toxic than the soluble drug) has been shown by LD50 and convulsive dose studies in mice. Lower tissue toxicity of microencapsulated lidocaine has been demonstrated by CPK analysis after injections of solutions and microcapsule suspensions in rabbits. The duration of anesthesia was measured by the blockage of the sciatic nerve of the rat using etidocaine-HCl microcapsules (150-212 μ m, 47% drug content, 50% *in vitro* release in 23 hours). Most blocks which lasted 12 hours also lasted 48 hours. The median effective dose for this anesthesia (ED50) was approximately 50 mg (150 mg/kg). The convulsive dose (CD50) was 330 mg/kg in this experiment. With etidocaine solutions 48 hours of anesthesia could not be achieved. One day blockage was achieved, but the dose was above the convulsive dose (ED50 > CD 50).

The contract on wound dressings has benefitted from the effort on microencapsulated anesthetics. The method of encapsulation is the same as for the microencapsulated anesthetics.

a. Microencapsulation of Povidone-Iodine

Povidone iodine fabric and powder composites did not release active iodine in a continuous manner. Composite powders have had segregated areas of drug and polymer. Most of the drug has been released very rapidly from these powders, at 20% drug loading. Non-woven fabric

material has held the drug more firmly. At 20% loading, almost no release was obtained, but at 40% a rapid partial release was observed.

To improve the active iodine release from the powders, these materials have been coated with a layer of polymer using the air suspension coating equipment, as used in Contract DAMD17-81-C-1195. This approach can theoretically yield zero-order drug release.

A composite of 80% drug and 20% polymer was prepared as an evaporated film from methylene chloride. This film was hard, but friable, and was coarsely ground with a mortar and pestle. This powder was then ground with a pestle on a 500 micron sieve. The resulting material was then ground through a 250 micron sieve. The final size distribution of this core powder is shown in Table 6.

This powder was coated with an additional 10% of polymer, and a representative sample was obtained from the bed (see Table 7). Coating was continued to 20% polymer and another sample was taken. At this point the bed contained more material than it did at the 10% loading. The coating operation was restarted and the final sample was obtained at 30% coating. A brush-down of the equipment yielded another 68 grams of product. The size distribution of the microcapsules is shown in Table 6.

b. Microencapsulation of Antibiotic Drugs

Recently a small microencapsulation unit was designed which could use as little as 10 grams of core material in a fluidized bed. This unit was used to microencapsulate chlorhexidine diphospanilate, ampicillin and clindamycin.

C. In Vitro Drug Release Studies

The measurement of drug release as a function of time was the primary measurement of this program. The standard method of analysis was to measure drug release into a large (40 ml) volume of phosphate buffer. In this procedure the sample was maintained at 37°C and agitated. In a secondary procedure, drug release was measured into a small (1 ml) volume of solution (buffer, serum, etc.) which was maintained at room temperature (21° + 3°C) without agitation. The preferred method of drug analysis was by spectrophotometry. Iodine was initially measured by thiosulfate titration, and later by the absorbance of the starch-iodine adduct. In a complex system (e.g., ampicillin release into serum), liquid chromatography (HPLC) was used with a spectrophotometric detector for the drug analysis.

Drug release rates are determined by suspending a known quantity of drug in a powder, microcapsule, or fabric form in a known volume of an aqueous solution and periodically measuring the concentration of the drug in the solution. From the solution volume and concentration, the quantity of drug released is computed. This quantity divided by the length of time since the last measurement is the average drug release rate over the time interval.

TABLE 6

PVP-I₂ (BASF 17/12) MICROCAPSULE SIZE DISTRIBUTION

(Values are % of weight in each sieve fraction)

Microcapsule Size Range (μ m)	20% Polymer Core	% Core in Microcapsules		
		90%	80%	71%*
> 600	0	0	1.2	4.4
425-600	0	0.2	12.0	17.7
300-425	0.9	2.7	31.7	35.0
212-300	37.2	64.0	26.5	17.4
150-212	18.6	14.4	11.4	6.8
106-150	15.6	7.3	8.4	6.5
75-106	11.8	5.7	4.8	6.5
38-75	10.1	5.6	3.9	5.6
< 38	5.9	0	0	0

* Does not include brush-down material

TABLE 1

PROCESSING SUMMARY OF PVP-I₂ (BASF 17/12) MICROENCAPSULATION

(Run 11-5)

Process Polymer %	Starting Weights		Final Weight		Losses			
	Starting Sample g	Polymer Added g	MC (as drug) g	(g)	Wurster Holdup g	Oversize Removed g (500 μ m)	Sieve + Bag g	Samples Removed g
0-10	230***	26	80	(58)	176	-	-	26
10-20	54	27	190	(122)	-109	-	-	32
20-29	158	23	131 (68)	(74) (38)	50 -68*	-	-	131 68*
TOTALS		76			49			257**

A B C D E F G

Material Balance: In: 184 g drug + 122 g polymer = 306 g
 Out: 257 g samples + 49 g Wurster holdup = 306 g

Yield at 30% is 61% based on drug [not including samples purposefully removed, (74 + 38)/18.4]**
 Yield at 30% is 84% based on samples/input materials [257/(230+76)]**

$$D_n = A_n + B_n - C_n$$

$$A_{n+1} = C_n - (E_n + F_n + G_n)$$

* Brush down of Wurster unit yields 68 grams

** Includes brushed-down material

*** Starting sample contains 20% polymer (46 g polymer and 184 g of drug)

In the standard 40 ml test system the suspending solution is water buffered with phosphate (pH 7.4 Sorenson's buffer, 0.05 molar). These solutions are thermostatted at 37°C in a metabolic shaker bath. The vessel used for the release studies is a specially designed L-shaped test tube. The shape of this container promotes good mixing of the release solution when used in a metabolic shaker and thereby reduces local drug concentration gradients in the solution which might affect the release rate. The polymer-drug composites are placed in a tea-bag type structure, constructed from fine polyester mesh for convenience in separating the fabrics and particles from the suspending solution.

Later in the program, BIOTEK installed a small computer facility and the in vitro diffusion data of this program has been entered on computer disk for ease of entry, storage, sorting, and graphing. We are using a LOTUS 1-2-3 worksheet program which is available for our IBM Personal Computer. This package also includes a GRAPH-program disk.

1. Anesthetic Drug Release

All of the anesthetic drugs were soluble in phosphate buffer, and relatively stable. They also had sufficient ultraviolet absorbance to be measured spectrophotometrically. At the suggestion of Astra technical personnel, etidocaine was acidified with HCl prior to the measurement. Bupivacaine was similarly acidified. Etidocaine and lidocaine were measured at 271 nm, lidocaine at 262 nm, and benzocaine at 278 nm.

Standard curves were generated from the pure drugs and a least-squares equation was used for setting the calibration curve. Calculations, involving assay values and sample discards were performed by computer. The computer program was written to accommodate drug diffusing into 40 ml of solution, removing 2.8 ml, acidifying with 0.2 ml of acid and discarding either the analyzed sample or the entire sample. Drug assays were performed in methylene chloride, using a calibration curve in the same solvent.

a. Lidocaine Release from Fabrics

Lidocaine-HCl (Sterling Drug) was incorporated into fabrics at 10, 15, and 20% drug. The lidocaine release was very rapid initially, with little continued release (32% in 1 hour and 52% within 24 hours for 15% drug fabric).

The base form of lidocaine was purchased from Sterling Drug and tested in fabrics at 10, 15, and 20% drug. Variable release was observed, which in hindsight may have been due to slow and incomplete wetting of this fabric. The best results were obtained with the 20% loaded fabric (36% in 1 hour, 78% in 6 hours, and 96% in 24 hours). A second fabric preparation released less drug, but still showed a continuous release (10% in 1 hour, 26% in 6 hours, and 38% in 24 hours). The effect of storage was studied on the initial 20% lidocaine-base fabric.

Lidocaine salts can be prepared by precipitation of the less soluble salt in water or by addition of the appropriate acid to the base form of the drug. Best results were obtained by dissolving the lidocaine (base)

in ethanol and adding concentrated sulfuric or phosphoric acid solutions (aqueous). The precipitates were then washed with ethanol. Lidocaine sulfate has a melting point of approximately 215°C and is slightly soluble in water. Lidocaine phosphate has a melting point of approximately 180°C, but it is very soluble in water. Thus the lidocaine sulfate was the salt of choice for fabric preparation. However no good fabric material was obtained with this lidocaine sulfate.

b. Lidocaine Release from Powders

Lidocaine-HCl powders were prepared at 5, 10, and 20% drug loading. Each powder was sieved into size fractions and selected sizes analyzed for drug release. The 212-300 um size fraction of 20% lidocaine-HCl powder showed some sustained release (66% in 1 hour, 81% in 6 hours, and 85% in 24 hours). This sample was used for stability studies. Based on the animal anesthesia data of Contract DAMD17-81-C-1195 using lidocaine microcapsules, we did not believe that a smaller quantity of lidocaine release would be effective for local pain control.

Lidocaine-base powders were prepared at 10 and 20% drug, but only the largest particles (300-425 um) showed a sustained release. At 20% loading 61% of the drug was released within 1 hour, 91% within 6 hours and all of the drug (103%) released in one day. This sample was also used for stability studies.

c. Etidocaine Release from Fabrics

Etidocaine hydrochloride and base were obtained from Astra Pharmaceuticals as courtesy samples. Fabrics were prepared at 10, 15, and 20% etidocaine-HCl and 10, 20, 40, 60, and 80% etidocaine base.

Release of etidocaine-HCl was slow from 10% fabric and faster from the 15% and 20% fabrics. The 20% fabric released 28% in 1 hour, 38% in 2 hours, 52% in 6 hours, and 65% in one day. A remake of this fabric showed less of a burst, but good continuous release for 6 hours. The initial sample was used to study the effect of storage conditions.

Etidocaine base was quite insoluble and was very slowly released from the fabric structures. However pure etidocaine base powder dissolved slowly in similar "drug release" studies (10% in 1 hour and 30% in 6 hours).

d. Etidocaine Release from Powders

Etidocaine-HCl powders were prepared at 10, 20, and 30% drug and etidocaine base powders were prepared at 20%. Etidocaine base powders at 20% loading released similarly to the pure drug crystals. Large particles of etidocaine-HCl at 10 and 20% loading showed sustained release (20% loading, 300-425 um, gave 24% release in 1 hour, 38% in 2 hours, 52% in 6 hours, and 61% in 24 hours). A 212-300 um fraction of the 30% etidocaine-HCl powder was saved to study the effect of storage conditions.

e. Bupivacaine Release from Fabrics

Bupivacaine-HCl was prepared for us by Chem Biochem Research, Inc. (Salt Lake City, Utah). The base form was prepared at BIOTEK by adding NaOH to an aqueous solution of the bupivacaine hydrochloride. The crystals were then filtered and washed with water.

Bupivacaine-HCl fabrics were prepared at 10, 15, 20, 30, and 50% drug loading. The initial release was influenced by the drug content, but only a slight continuing drug release was seen. At 30% loading 18% of the drug was released in one hour, but only 31% was released in one day.

Bupivacaine base was dissolved slowly as pure crystals in the drug release system. Fabrics at 20, 40, and 60% drug were very slow to release their bupivacaine to the agitated buffer solution.

f. Bupivacaine Release from Powders

Bupivacaine-HCl was used to make powders at 10 and 20% drug loading. There was an immediate release from even the largest particles of 10% drug.

Bupivacaine-base powders were prepared at 20% drug loading. The release was similar to that of the pure drug crystals (See Figure 3).

g. Benzocaine Release from Fabrics

Benzocaine (ethyl-p-aminobenzoate) was obtained from Sigma Chemical Company. Fabrics were prepared at 10, 15, 20, and 30% drug loading. Even at 30% loading the drug release was slow and incomplete in one day (12% in 1 hour, 20% in 6 hours, and 31% in 24 hours). A 20% fabric gave similar release and was stored for stability studies.

h. Benzocaine Release from Powders

Benzocaine powders were prepared at 10, 20, and 30% drug. The largest particles of the highest loading gave the best sustained release (30% loading, 300-425 um, 14% release in 1 hour, 24% in 6 hours and 34% in one day). A 20% powder of 212-300 um size was maintained for stability studies (41% release in 1 hour, 51% in 6 hours, and 59% in one day).

Fabric samples were placed on clean glass slides which have wells bounded by 1 mm high ceramic rings of 1.2 cm diameter (Fisher Scientific, 12-568-30). Various solutions (0.15 ml) were carefully added with a syringe and wetting was observed visually. Solutions of water, buffer, rabbit serum, 0.2% sodium lauryl sulfate and 0.005% alkyl amine hydrochloride (Hyamine™ 2389) were tested. Only the sodium lauryl sulfate wetted the fabric. A sample of 40% povidone iodine fabric was wetted even with pure water by this test method. Nitrofurazone release was studied into these small reservoirs using water, buffer, and 0.014% sodium lauryl sulfate (SLS) as the wetting and drug release media. Fabric samples (approximately 2 mg) of 20% nitrofurazone were placed in 150 ul of the solution. At hourly intervals 100 ul of the solution was collected for analysis. The sample was then transferred to a new test well and rewetted with 150 ul of the test solution.

Fabrics in the SLS reservoir released 4 to 5 times more drug per hour than fabrics in the water or buffer reservoirs. However reproducibility was not improved. The amount of nitrofurazone released into these SLS wells was comparable to that released by the larger swatches of fabric (13 mg) into 40 ml of 37°C buffer in a shaking system in which the fabric is enclosed in a polyester mesh and submerged in the buffer solution. The data are presented in Table 9.

In addition to demonstrating the wetting problem, this type of test is also more representative of the use of a wound dressing. The fabric must be wetted by the tissue fluid in a static environment with a small amount of downward external pressure which is applied by the bandage. Wetting of the fabric or powder in this environment may be a significant problem. Secondly, concentration gradients within the fabric or powder mass may lower the drug release rates from that observed in a shaking system. This is especially important with drugs which could saturate the local, absorbed solution.

2) Powder

Suspensions of nitrofurazone in polymer solutions in methylene chloride were prepared and cast on glass plates. The drug was suspended in viscous 20% polymer solutions, and there appeared to be very little settling prior to solvent evaporation. These films were broken into pieces which could be fed through the hammer mill with dry ice. The mill was assembled with a 20 mesh classifier screen. The machine was pre-cooled with about 500 grams of dry ice and then the sample (approximately 25 grams) was sent through with 10:1 dry ice:drug-polymer. A final 100 grams of dry ice was sent through the equipment to increase the product yield (decrease hold-up).

Films of 10 and 40% nitrofurazone in polylactide were prepared and ground. Unfortunately the 20% sample was contaminated with polystyrene which entered the grinder with the dry ice. The size distribution of the samples is shown in Table 10.

Selected sizes of 10 and 40% nitrofurazone powders were analyzed for their drug release characteristics in in vitro tests. As expected, the results (Figure 10) show significant decreases in the rate of drug diffusion with increasing particle size. Smaller rate differences were

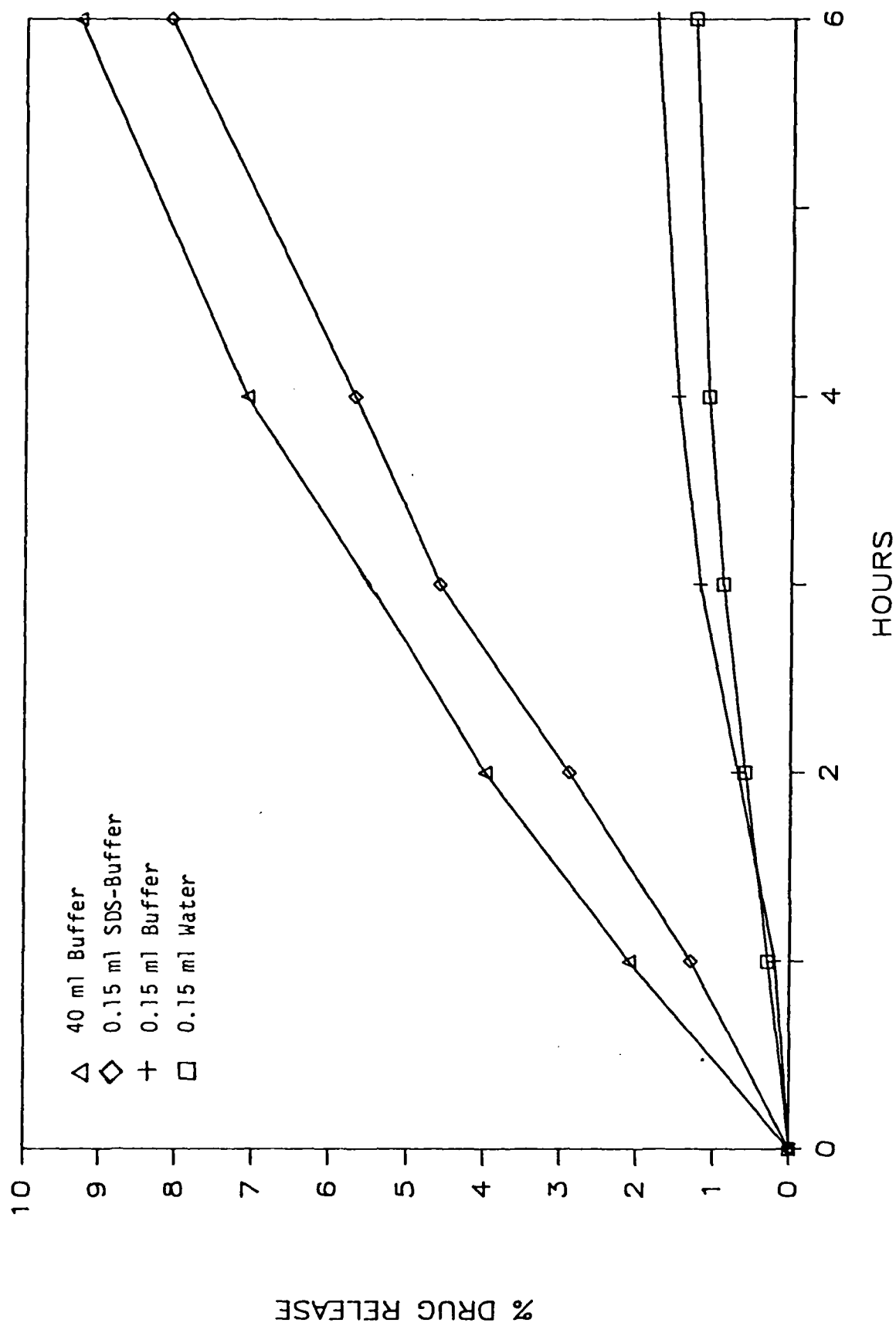


Figure 8 AVERAGE IN VITRO NITROFURAZONE RELEASE FROM FABRICS

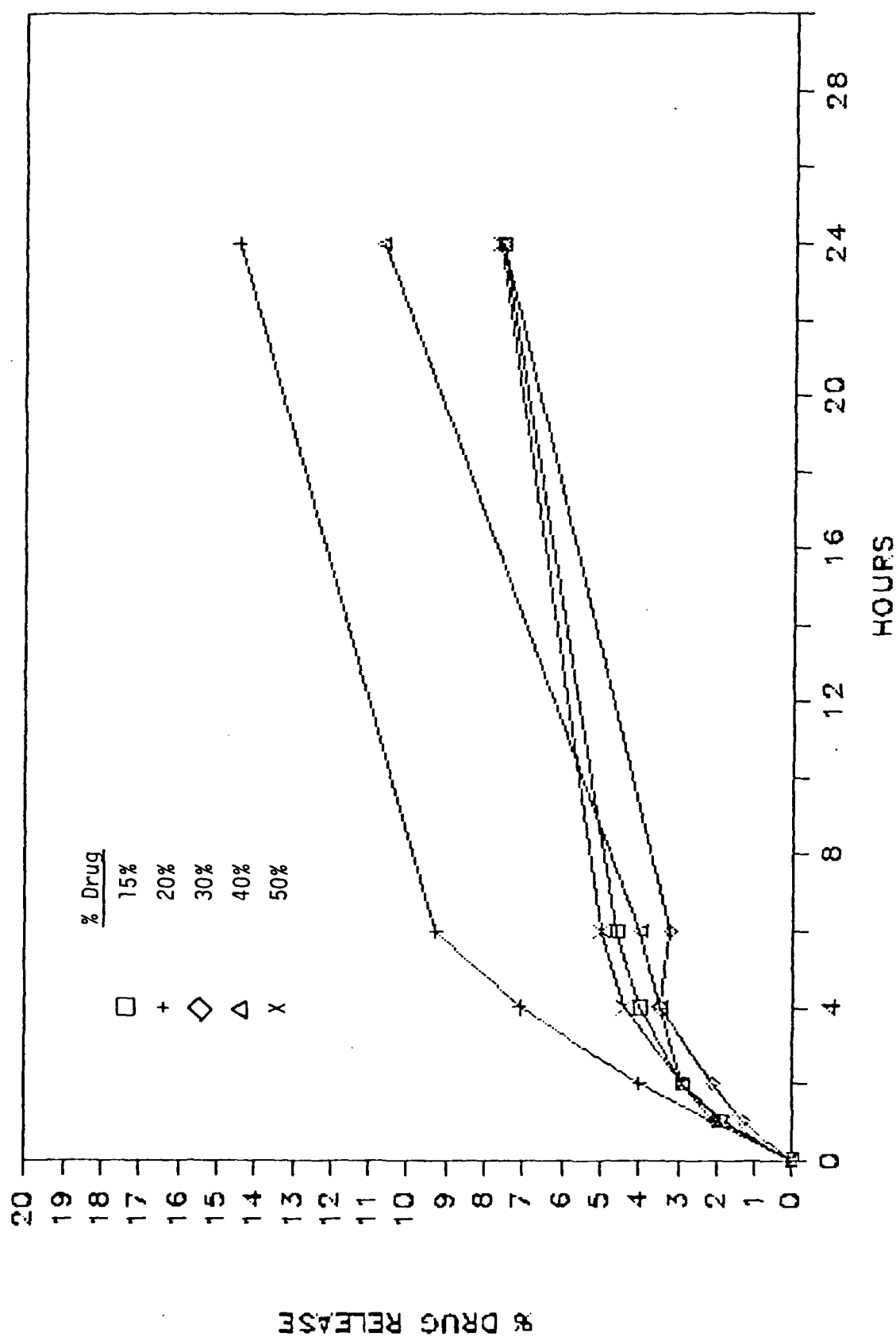
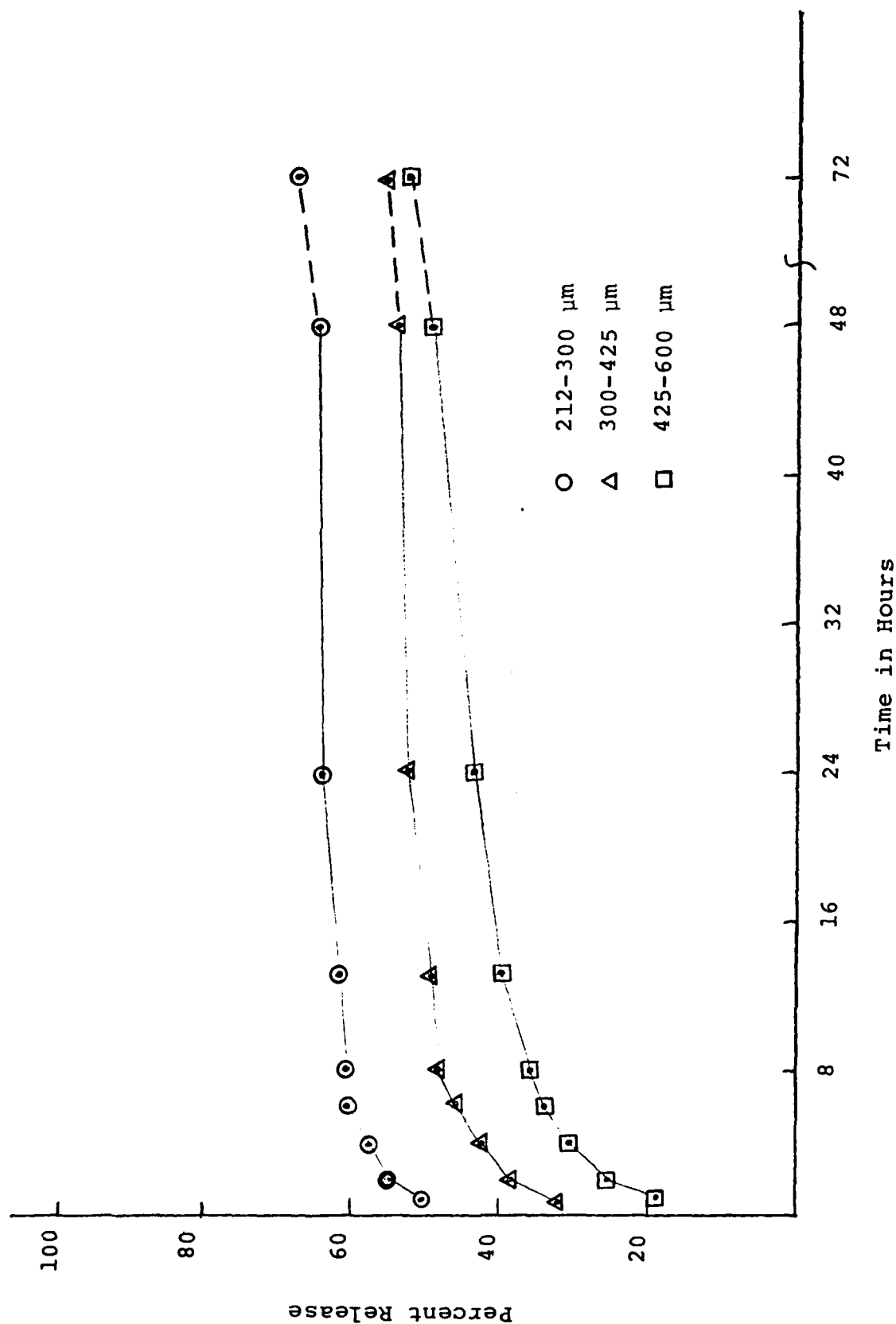


Figure 7 Release of Benzalkonium Chloride from 13% Drug Powders
of Various Sizes



per square centimeter. About 30% of this drug is released immediately, which is 600 ug/square centimeter. Since this is at least 10 times too much, for use as an antiseptic, we considered methods to spray a mixture of fibers in order to prepare a wound dressing which will release drug in a manner similar to the 20% fabric.

2) Powders

Homogeneous powders were prepared with 20% Maquat LC-12S. Surprisingly the analysis of all the sieve fractions were $13.0 \pm 1.0\%$ based on the ultraviolet absorbance which is used for the assay and drug release method. There was no significant continuous drug release from the small powder samples. The drug release from the coarser powders is shown in Figure 7. Significant continued release is observed from particles of approximately 0.5 mm in diameter. In all cases (6) the residual BAC was $37.0 \pm 3.9\%$. This was unavailable drug from either small or large particles. The 425-600 um sieve fraction was stored for stability testing.

3) Microcapsules

Because of the limited potency of this drug no microcapsules were prepared with benzalkonium chloride.

c. Nitrofurazone Materials

Nitrofurazone was graciously supplied to us by Norwich-Eaton Pharmaceuticals.

Nitrofurazone is practically insoluble in methylene chloride and no combination of methanol and methylene chloride was found which could dissolve both the drug and polymer. Nitrofurazone was received as a very fine powder and it was readily suspended in methylene chloride. Therefore both fabrics and powders were prepared with a suspension of drug in this polymer solution. Nitrofurazone is measured spectrophotometrically at 260 nm in buffer, and at 385 nm in dimethylformamide for the assay values of the composites.

1) Fabric

Nitrofurazone fabrics were prepared by the conventional technique at 20% drug loading. Good cohesive non-woven fabric was collected. Drug release was slow as shown in Figure 8. Although nitrofurazone dissolves slowly in water, 90% dissolved in one hour when the pure drug powder was placed in a diffusion cell.

Although the results of drug release from this fabric were variable, we proceeded to make additional fabrics at 15, 30, 40, and 50% nitrofurazone loadings. We expected that the fabrics with more drug (per polymer weight) would release their drug more rapidly. Fabric was formed, even at 50% drug loading, but the drug release was variable at all drug loadings (Figure 9). Incomplete wetting of the sample may be the reason for this variation. A wetting problem had been shown previously with povidone iodine fabrics. Wound exudate or serum may or may not wet these fabrics better than aqueous buffers.

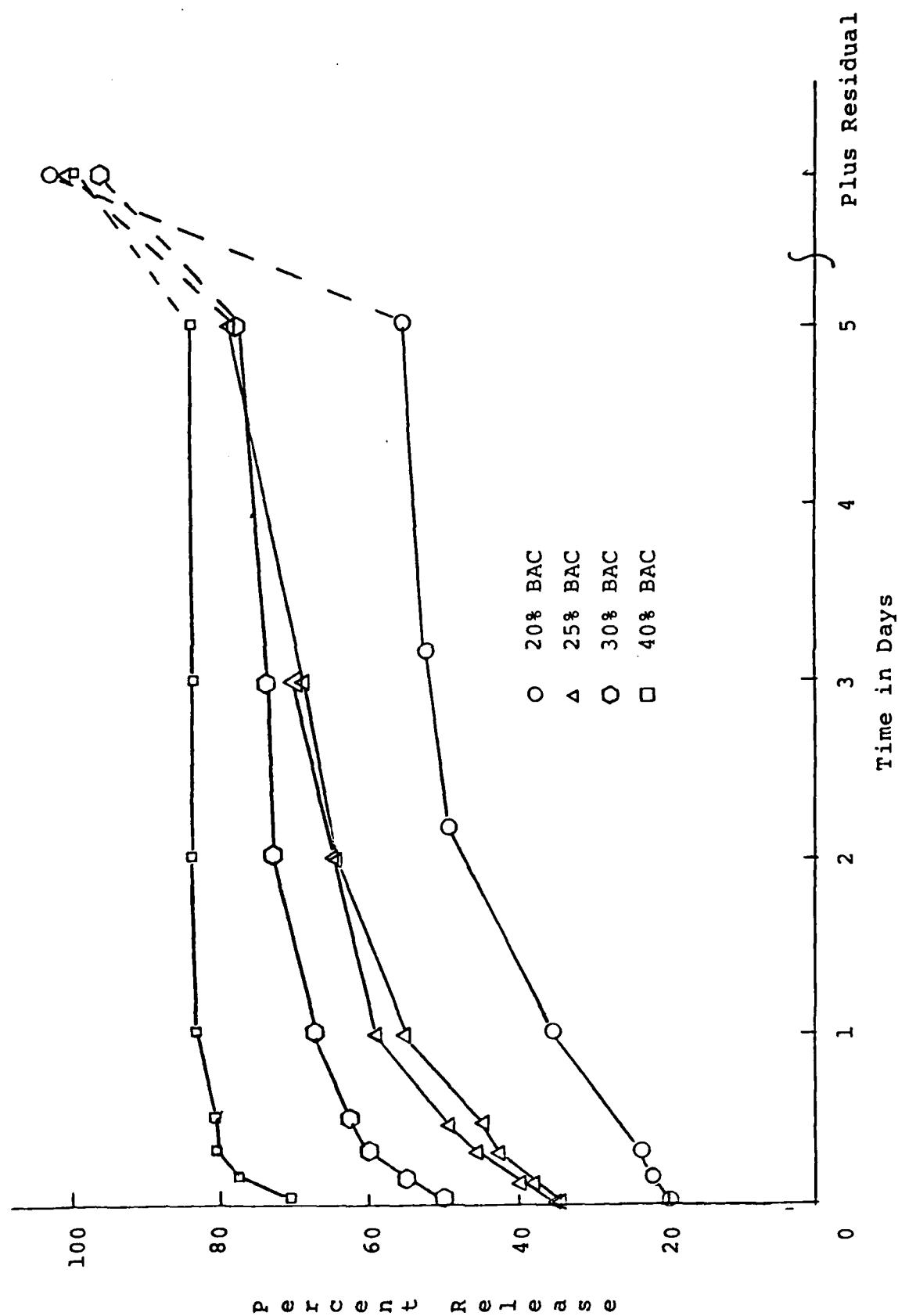


Figure 6 Release of Benzalkonium Chloride (BAC) from Polylactide Fabric

TABLE 8

Calculation of Reducing Groups in Serum and
Reaction Rate Constants With PVP.I₂

$\frac{a}{\text{mg/ml}}$ PVP-I ₂	$\frac{1}{\text{minutes}}$	if $\frac{b}{22}$ then k_1	if $\frac{b}{25}$ then k_2
20	335	0.0072	0.00050
15	187	0.0061	0.00053
10	103	0.0059	0.00065
5	32	0.0081	0.00156
<hr/>		<hr/>	<hr/>
average		0.00682	0.00084
\pm S.D.		\pm 0.00102	\pm 0.00048

This equation integrates to
$$\ln \frac{b-a}{b} = -k_1 \tau$$

One can then calculate values for this rate constant k_1 for various values of b and choose the solution which is most consistent (k_1 is a constant). There is also some effect on this calculation by choosing different redox potentials for the end point. By choosing 190 mv for the end point the most appropriate value for b is 22 (mg as povidone iodine). This is equivalent to about 0.017 meq/ml of reducing groups in serum, based on an equivalent iodine content of 10% for BASF 17/12 povidone iodine. The rate constant is 0.0068 ± 0.0010 minutes--based on the four data points (See Table 8).

Although this analysis gives the sought logarithmic relationship and a constant apparent rate constant, we could find no reference to this approach in standard texts. A second order dependence is probable for this experiment and the appropriate equation is

$$\frac{1}{a-b} \ln \frac{b(a-x)}{a(b-x)} = k_2 t,$$

assuming the same end point conditions.

$$\frac{1}{a-b} = k_2 \tau$$

Again we can find b by reiteration, such that k_2 is constant. This reaction hypothesis fails at low iodine concentrations (a), but gives a second order rate constant K_2 of $1.3 \text{ min}^{-1} \text{ meq}^{-1}$ and a serum reducing concentration of 0.020 meq/ml (See Table 8).

These analyses may be useful in determining the quantity of povidone iodine required to maintain an oxidizing environment in the wound area for various periods of time. However the contract efforts were then directed towards antimicrobials which have higher activity and stability in the presence of serum components.

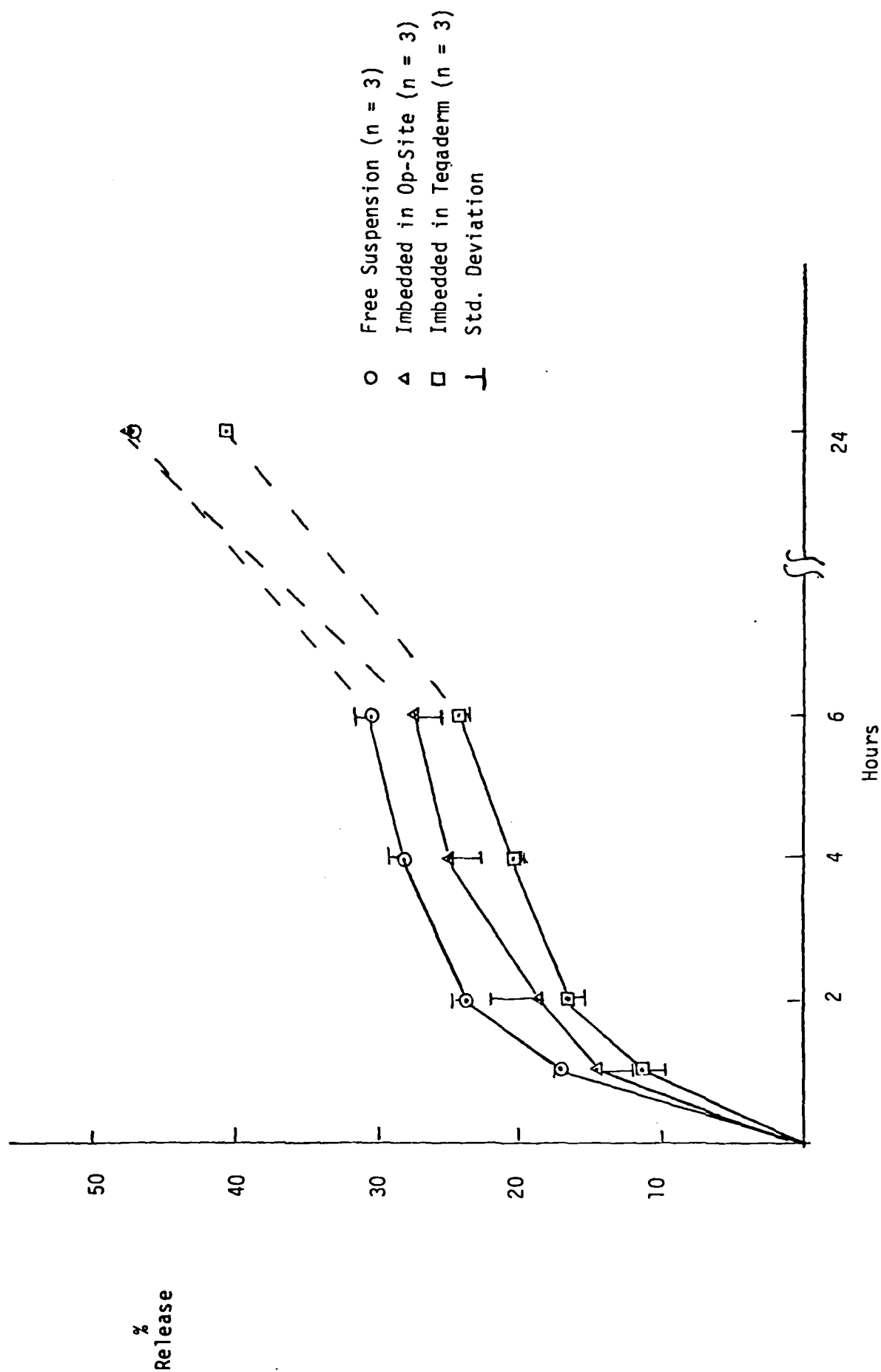
b. Benzalkonium Chloride Materials

Benzalkonium chloride is a stable chemical which is readily measured at 262 nm in buffer and methylene chloride. It is also bacteriostatic at low concentrations.

1) Fabrics

Fabrics were prepared with 5, 10, and 20% benzalkonium chloride (Maquat LC-12S). Practically no continued drug release was obtained with 5 and 10% loading of the drug (BAC), but about 15% of the drug was released immediately. Samples were then prepared at 20, 25, 30, and 40% drug; the resulting drug release is shown in Figure 6.

Our standard fabric uses 50 ml of solution and sprays a fabric of about 10 mg/square centimeter. At 20% drug, we would have 2 mg of drug

Figure 5 In Vitro Release of PVP-I₂ from Microcapsules (11-5-30)

distribution of microcapsules over the adhesive surface. Drug release studies were run with microcapsules imbedded in Tegaderm™ and in Op-Site™. These data are shown in Figure 5 and compared with the release of freely dispersed microcapsules.

4) Povidone Iodine Decomposition

In *vitro* experiments with povidone iodine have been modified to minimize the effect of iodine loss. However there would be advantages to monitoring both the iodine and iodide in solution as povidone iodine is being released from fabrics, powders, and microcapsules. Therefore an iodide and a redox electrode were purchased from Orion Research, Inc., of Cambridge, Mass.

In a preliminary experiment, povidone iodine was titrated with sodium thiosulfate in water and the redox electrode potentials were compared to the visual starch end-point. A typical potentiometric titration curve was obtained. The visual end-point would be chosen at 304 mv; whereas the inflection point of the potential titrant curve was around 270 mv (7.8 vs. 8.0 ml of titrant). Tests with povidone iodine in serum were inconclusive when we titrated a povidone iodine solution with human serum. There was an indication of a slow rate of reaction between povidone iodine and serum proteins.

A series of experiments were then performed to determine the length of time that the active iodine would be present in serum samples, based on the original concentration of povidone iodine in rabbit serum. This information would be valuable in determining the concentration of povidone iodine to be used in a wound to maintain bacteriostatic effectiveness for a given period of time, assuming the povidone iodine was applied as a soluble dressing. It might then be possible to calculate the rate of release needed to maintain this active iodine potential when using a slow release formulation.

Potential time curves were obtained for various concentrations of povidone iodine in serum and the time increased as the initial povidone iodine concentration increased.

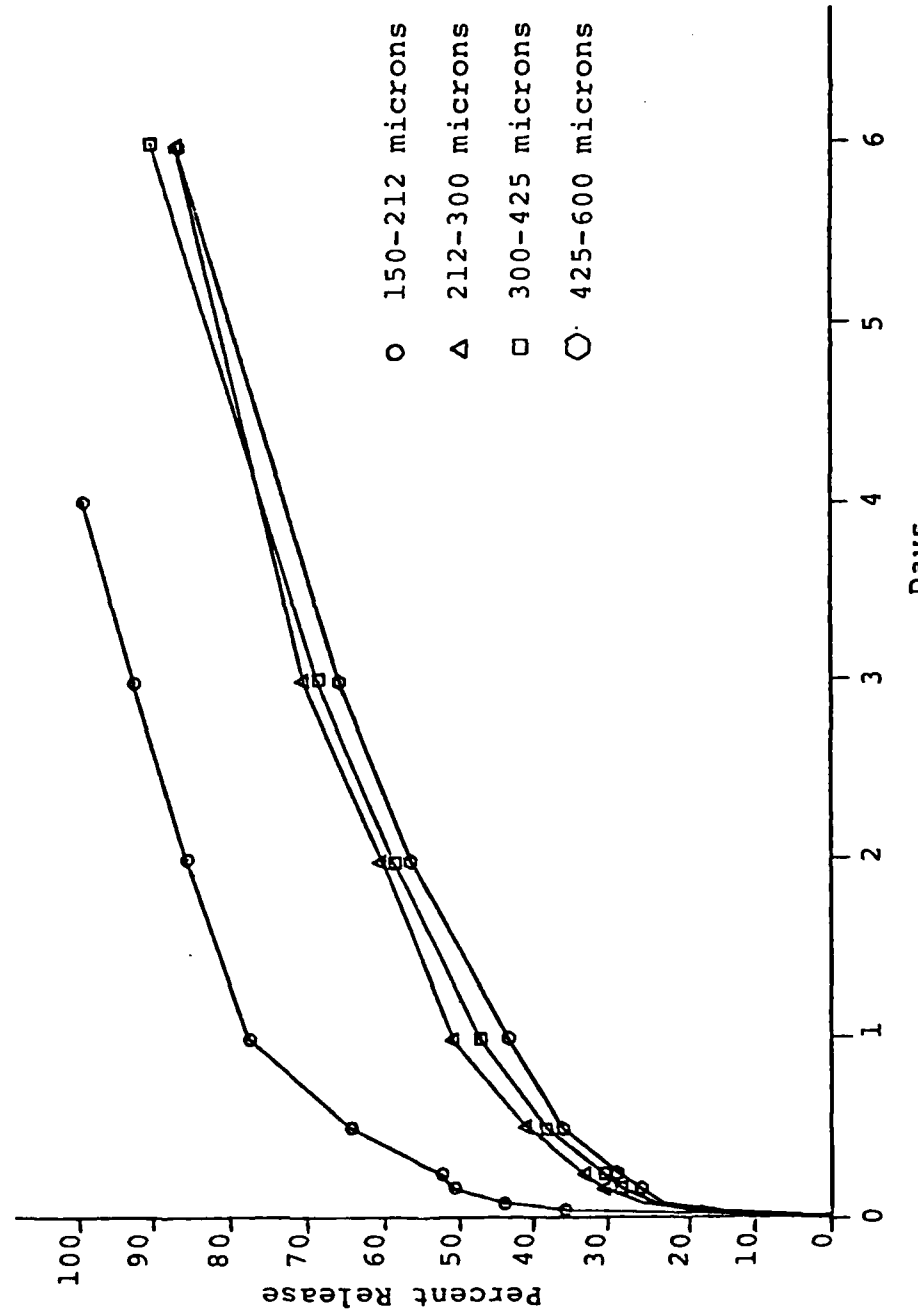
If one considers the reaction of iodine with a serum component (e.g., sulfhydryl groups) in which the rate is first order with respect to the remaining serum reducing groups, then:

$$\frac{d[I]}{dt} = -k_1[b-(a-[I])] \quad \text{and}$$

$$\int_{I=a}^{I=0} \frac{d[I]}{b-a+[I]} = -k_1 \int_{t=0}^{t=\tau} dt$$

where a is the initial povidone iodine concentration, b is the initial concentration of serum reducing groups, and τ is the end point as the concentration of active iodine approaches zero ($b > a$).

Figure 4 Active Iodine Release From PVP-I₂ Microcapsules
with 30% Polymer Coating (11-5-30)



<u>Time (hours)</u>	<u>Release (%)</u>
1	37
2	53
4	62
6	70
24	92

The sample was 75% drug and 25% polymer. However, with about 75 mg of fabric, approximately 90% of the drug was released in one hour.

Samples were then prepared with 20, 30, and 40% povidone iodine in polylactide. Duplicate samples were tested, with and without 0.002% sodium lauryl sulfate as a wetting agent. Since there was no obvious effect of the surfactant, the results are averaged. There was an indication of a continuous release of active iodine at 20 and 30% drug and more continuous release of drug from the 40% fabric. There is also a problem when comparing the assay procedure in aqueous and non-aqueous media. This gives an unreasonably low assay and residual value with respect to the aqueous release data.

It may be unnecessary to have a slow release of povidone iodine. Iodine kills bacteria instantly on contact (Bogash, 1956), and povidone iodine releases active iodine within milliseconds after dissolution (Schlezinger, Mahinka, and Levin, 1979). Once a wound is antiseptic and bandaged, continued release of iodine may be unnecessary. However, it is imperative that active iodine be released from the wound dressing.

2) Powders

Povidone iodine was incorporated into powders at 20% drug loading. Some continued release was observed with the large sieve fraction, and the 300-425 μ m fraction was used for stability testing. Assuming a 15% drug content, 72% of the drug was released in 1 hour from this size fraction. An additional 7% was released in the next 5 hours.

3) Microcapsules

Iodine release was measured from the starting material and from microcapsules of 10, 20, and 30% polymer coating. The best results were with 30% coating, and these are shown in Figure 4. This data was the result of measurements in triplicate. The repeatability of the method is excellent as shown by the average of the standard deviations for the various time periods. The results were less precise for the smallest microcapsules. A sieve fraction of 212-600 microns would be 70% of the product (see Table 6). This material was sent for in vivo studies and stored for stability studies.

The microcapsules of povidone iodine (11-5-30, 212-600) adhered well to the contact adhesive used in Tegaderm™ and Op-Site™. Approximately 20 mg of microcapsules could be imbedded per square centimeter of surface area of either bandage. Slightly more microcapsules could be imbedded if a rolling pressure was applied to the microcapsules between successive applications of microcapsules. This quantity of microcapsules approximates a monolayer of capsules. Microscopic examination showed an even

2. Antiseptic Drug Release

For the antiseptics and antibiotics, three forms were possible. These were fabrics, powders and microcapsules.

a. Povidone Iodine Materials

Various iodine complexes were originally proposed, since slow release of a polymeric iodine complex might not diffuse through polylactide. Low molecular weight polyvinylpyrrolidone (PVP) and povidone iodine materials were requested from various suppliers. The original povidone iodine was prepared with a high molecular weight PVP (K30, Napp Chemicals, Inc.). For excretion by the kidneys, a lower molecular weight is advised for internal use, such as open wounds (K17, Schlezinger, Mahinka, and Levin, 1979). However, lower molecular weight povidone iodine is less stable to iodine loss (e.g. after 14 days at 52°C there is 6% loss with K30, and 12% loss with K17). For most of the work on this program a BASF sample of povidone iodine 17/12 was used (K17, M.W. ave. approximately 11,000, effective iodine content approximately 10%).

Various monomeric pyrrolidone iodine complexes were described by Schenck, Simak and Haedicke (1979). An attempt to prepare a methylpyrrolidone-iodine adduct by their method was unsuccessful.

Initial studies with iodine and povidone iodine were variable because of the loss of iodine from the solution, probably by escape to the atmosphere. Addition of starch and iodide to the buffer successfully trapped the iodine. This solution could be titrated with thiosulfate to a clear endpoint, or read directly on a spectrophotometer at 574 nm. For the study of the microcapsules, the drug release was conducted at room temperature to further decrease the loss of active iodine. Also the drug release was measured photometrically.

1) Fabrics

Fabrics were prepared with povidone iodine at 10, 20, 30, 40, 50, and 75% drug. Because of the polymeric nature of the drug, good fabric was obtained even at high drug loadings. At low drug loadings very little drug was released.

Some of the povidone-iodine fabrics were tested for drug release and the bags were opened and examined after 24 hours of release. Some sections of the bags contained clear (spent) polymer-PVP. Other areas of the bags were dark blue (iodine-starch product). In the center of this dark section was an area of orange material which turned dark blue when it was wetted with the release medium (KI-starch-buffer). This orange section appeared to be non-wetted fabric. The non-wetted fabric was found in the diffusion bag with the largest quantity of fabric. This slow wetting phenomenon leads to a slow release of drug from the non-woven fabric. However, it is very dependent on the thickness of fabric and wetting ability of the diffusion medium. In this one test with 100 mg of fabric in the 2 x 2 cm bag (approximate), the drug release was encouraging:

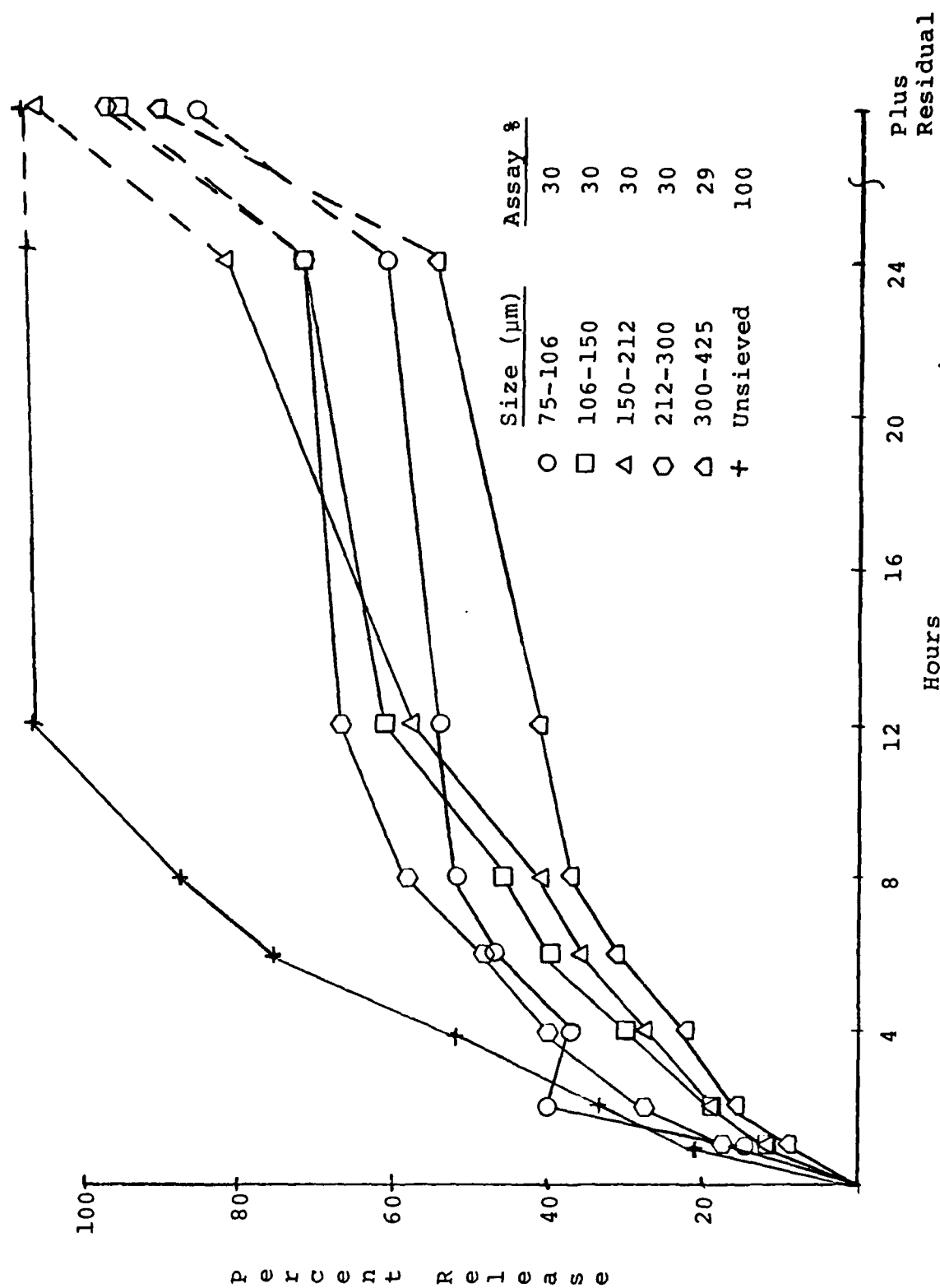


Figure 3 Bupivacaine (base) Release into Buffer of 20% Drug in Polymer Powder Samples and Pure Drug

TABLE 9
CUMULATIVE DRUG RELEASE FROM 20%
NITROFURAZONE FABRIC

A. Release into 150 μ l Reservoir (static, 22°C)

<u>Solution</u>	<u>n</u>	<u>Value</u>	% Release in Specified Hours				
			1	2	3	4	6
Water	2	Mean	0.3	0.6	0.9	1.1	1.3
		Range	0.0	0.0	0.2	0.6	0.8
Buffer	2	Mean	0.25	0.75	1.2	1.5	1.8
		Range	0.10	0.10	0.3	0.4	0.6
SLS	2	Mean	1.3	2.9	4.6	5.7	8.1
		Range	0.4	1.3	2.0	2.8	5.1

B. Release into 40 ml Reservoir (shaking, submerged, 37°C)

Buffer	3	Mean	2.1	4.0	7.2	-	9.3
		S.D.	0.4	1.1	2.2	-	3.1

SIZE DISTRIBUTION OF NITROFURAZONE POWDERS

(Data is % of Total Milled Sample)

<u>Size (um)</u>	<u>Drug in Powder</u>	
	10%	40%
600	9.6	2.4
600-425	13.8	4.1
425-300	20.5	10.3
300-212	19.4	19.9
212-150	14.4	20.9
150-106	12.3	21.1
106-74	6.1	11.4
74-38	3.2	7.7
· 38	0.0	0.0
Percent Yield of Milling	81	81

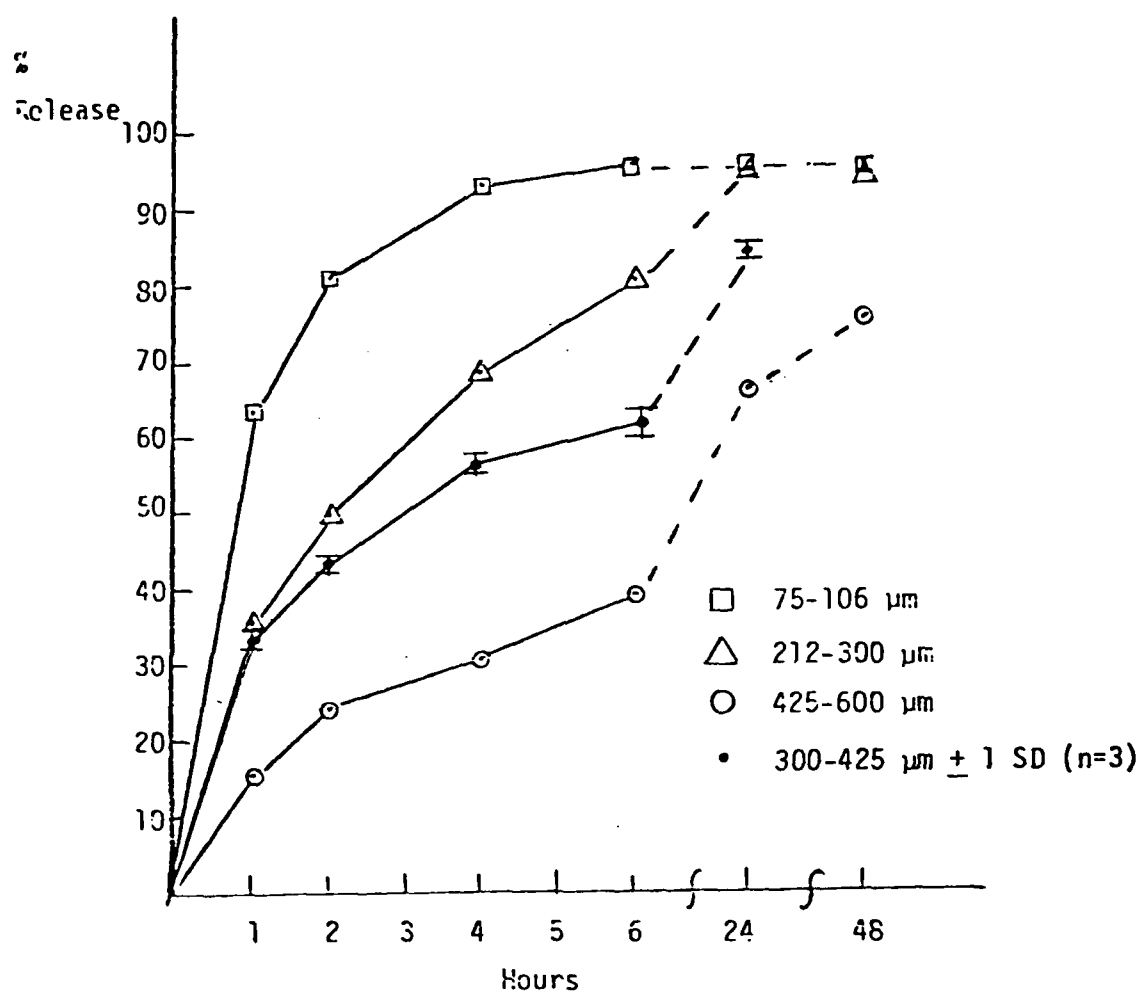


Figure 10 IN VITRO RELEASE OF NITROFURAZONE FROM POWDERS OF 40% DRUG

observed for the same size particle at different drug loadings. Large variations in the rate of drug release can thus be achieved with these powders, and the 300-425 um sieve fraction was sent to USAIDR for animal studies. A sample has also been set aside for stability studies.

3) Microcapsules

Since the nitrofurazone powder at 40% loading delivered the drug at the target rate (i.e. 50% release in approximately 3 hours) and could be varied by a change of particle size, microencapsulation of this drug did not appear to be warranted. A higher drug loading might be possible with microcapsules, but the maximum loading of the powder might also be increased.

d. Chlorhexidine Diphosphanilate Materials

Chlorhexidine diphosphanilate was obtained as a courtesy sample from Westwood Pharmaceuticals (Bristol-Myers).

The uv spectrum was compared with a sample of chlorhexidine diacetate and it was determined that both chlorhexidine and phosphanilate absorb strongly at 240 nm. However there was sufficient overlap to make the measurement at two wavelengths and a calculation of both the chlorhexidine and phosphanilate ions impractical.

Chlorhexidine is a strong complexer and precipitates in phosphate buffer, in rabbit serum and in the presence of dodecysulfate ions. At the present time drug release has been measured into pure water and into a dilute quaternary ammonium surfactant solution (HyamineTM). In these media chlorhexidine and phosphanilate ions are released at the same rate to preserve electroneutrality.

Chlorhexidine diphosphanilate did not dissolve in methylene chloride, but the suspension in a viscous polymer solution was used to make the fabric and powder.

1) Fabric

A thick fibrous mat of 20% chlorhexidine diphosphanilate was sprayed without incident. Two samples of this fabric were used in the standard (40 ml) diffusion cell with water. Although reproducibility was poor, there was continuous release (see Figure 11, 4% in 1 hour, 15% in 6 hours, and 40% in one day). When using Hyamine the results were remarkably similar (5% in 1 hour, 12% in 6 hours, and 30% in one day). When using a small cell without agitation (1 ml), very little drug was released into either water or Hyamine (4% in 1 day).

2) Powder

Chlorhexidine diphosphanilate was cast at 5 and 20% loading into films and then milled into powders. Only the 20% powder demonstrated continuous release into 40 ml of water. The release was dependent on the particle size, as shown in Figure 12.

FIGURE 11

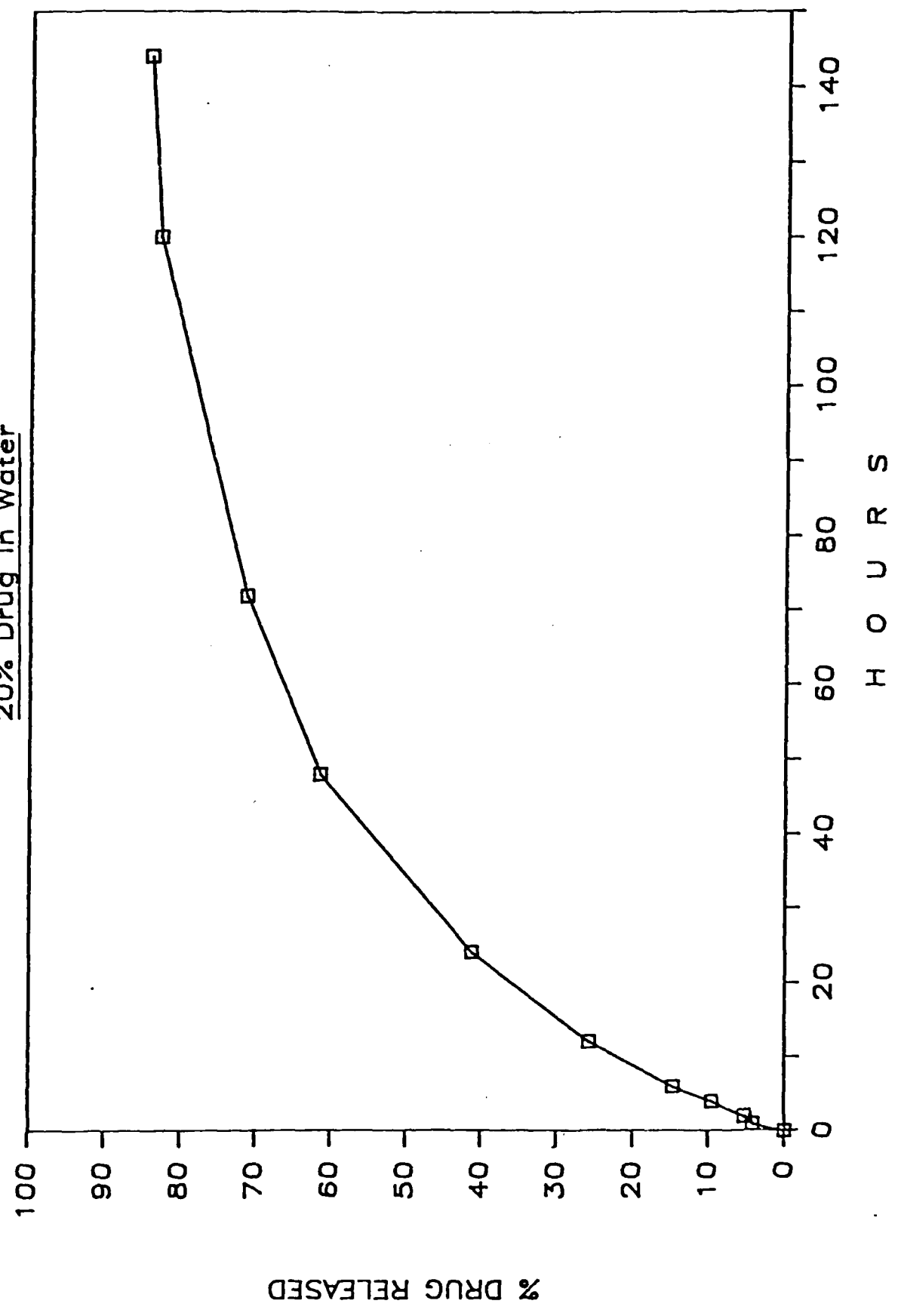
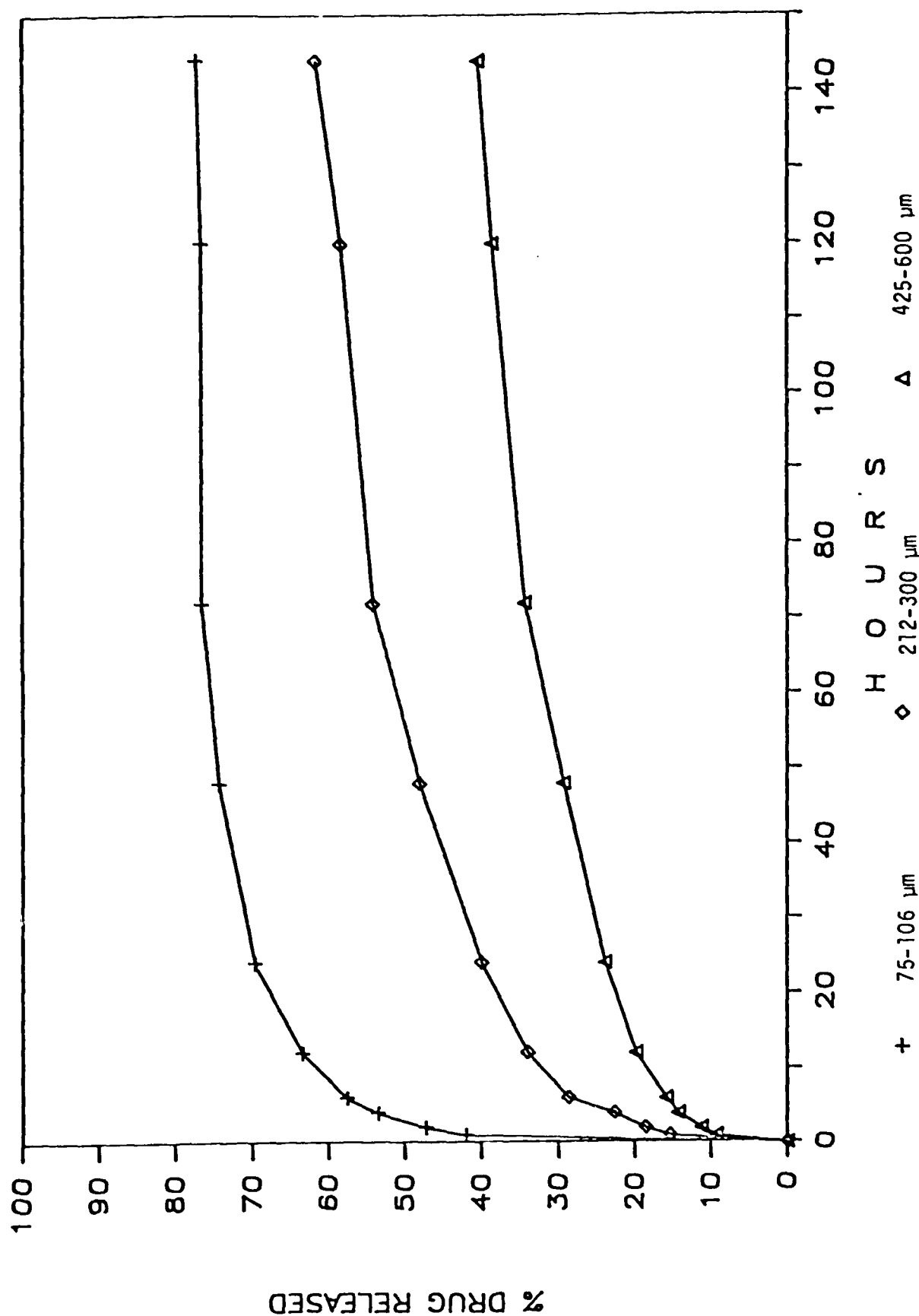
CHLORHEXIDINE DIPHOSPHANILATE FABRIC RELEASE20% Drug in Water

FIGURE 12

CHLORHEXIDINE DIPHOSPHANILATE POWDER RELEASEINTO 40% m1 OF WATER (20% DRUG)

The 20% chlorhexidine diphosphanilate powder of 212-300 um sieve size was also tested in the 1 ml cell. The drug release rate significantly slower in this test cell (12% release in 6 hours).

3) Microcapsules

Microcapsules of chlorhexidine diphosphanilate have been recently prepared using the small coating chamber (2 inch diameter). Approximately 11 grams of core material was coated to approximately 31% using a 2% polylactide solution. A nominal coating of 31% was obtained and a small sample was taken at about 14% coating. The sieve analysis of the 31% coated microcapsules is shown in Table 11. More than 90% of the microcapsules were larger than 210u. The greatest recovery was in the 600u and greater range.

The rate of diffusion of antiseptic from the microcapsules was determined at 37°C in 40 ml L-tubes containing 17 mM phosphate buffer pH 7.4. For large microcapsules, greater than 300u (Figure 13) the release rate approximates zero order after an initial rapid phase and continues for more than 700 hours. Microcapsule smaller than 300u (Figure 14) released chlorhexidine diphosphanilate on a first order basis. The release rate continued to decline until nearly 100% of the drug was released at 700 hours.

3. Antibiotic Drug Release

Antibiotics are considerably more labile than the anesthetics and antiseptics which have been tested on this contract. Also they tend to be less soluble in organic solvents and less absorbent in the near ultraviolet range of the spectrum.

a. Ampicillin Materials

Ampicillin absorbs more strongly in the uv than does clindamycin, although neither compound shows a uv maximum in the usual instrumental range. At 240 nm ampicillin absorbs strongly enough to be used for in vitro drug release and assay studies (392 ug/ml-AU for the sodium salt, 406 ug/ml-AU for the trihydrate and 324 ug/ml-AU for anhydrous ampicillin in pH 7.4 buffer). The purity of the anhydrous ampicillin is also questionable based on an HPLC peak for this material which was not present in the ampicillin trihydrate sample.

1) Ampicillin Fabrics

Ampicillin comes in variety of forms from several manufacturers. The trihydrate, anhydrous material, and the sodium salts are all insoluble in methylene chloride. However, the trihydrate (Bristol) and anhydrous material (Sigma) are small particles which are readily suspended in methylene chloride. Fibrous products are formed by spraying. Sodium ampicillin (Bristol) is a larger crystalline substance which does not suspend nor spray well. The product was a thin powdery mat. A lyophilized sodium ampicillin has been ordered from H. Reisman Corporation.

TABLE 11

SIZE DISTRIBUTION OF CHLORHEXIDINE
DIPHOSPHANILATE MICROCAPSULES

<u>Microcapsule Size μm</u>	<u>% Weight</u>
Fines	0.1%
74-105 μm	0.2%
105-149 μm	1.3%
149-210 μm	4.4%
210-300 μm	10.0%
300-425 μm	25.8%
425-600 μm	26.8%
>600 μm	31.3%

CUMULATIVE RELEASE

CHLORHEXIDINE DIPHOSPHANILATE

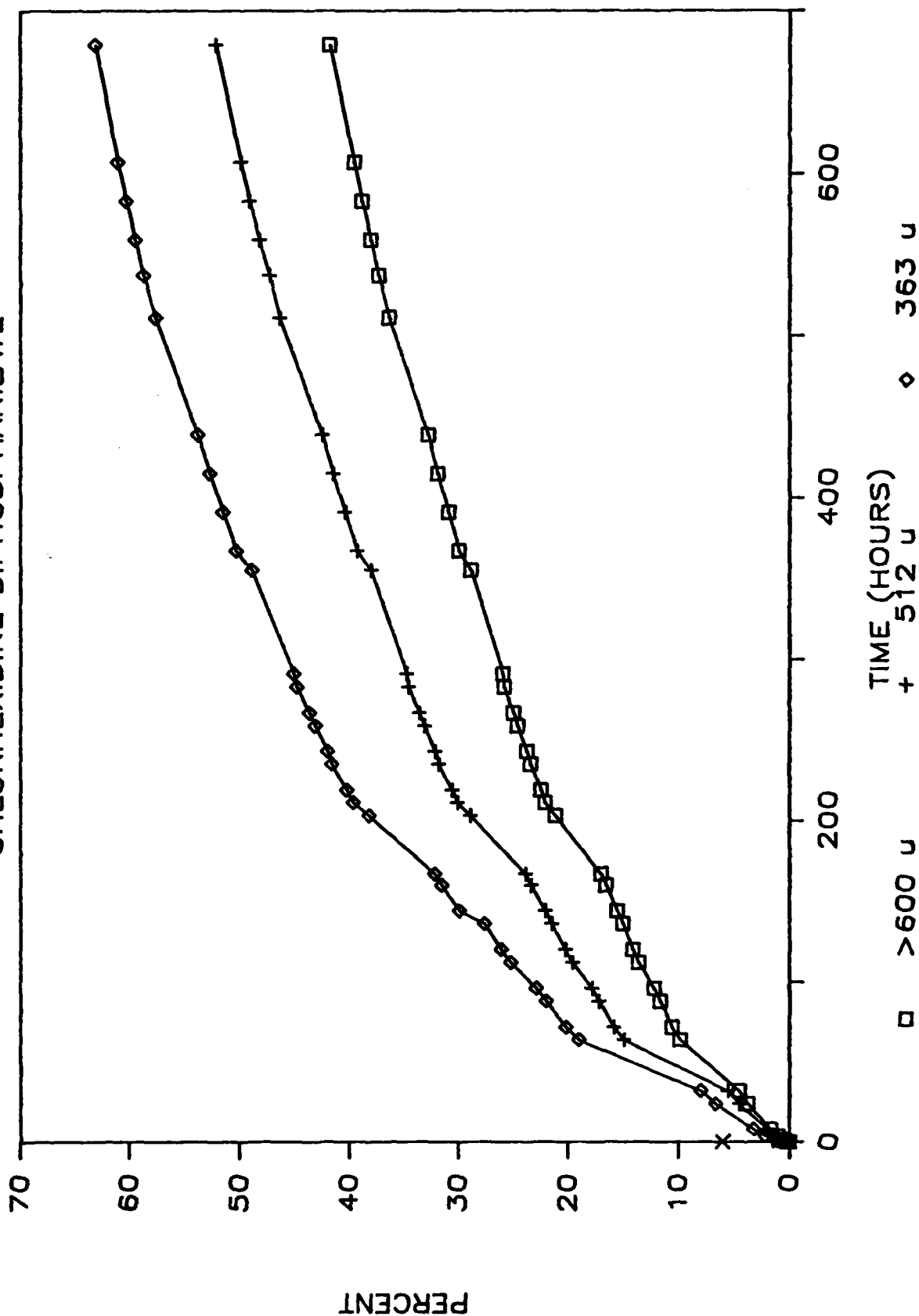
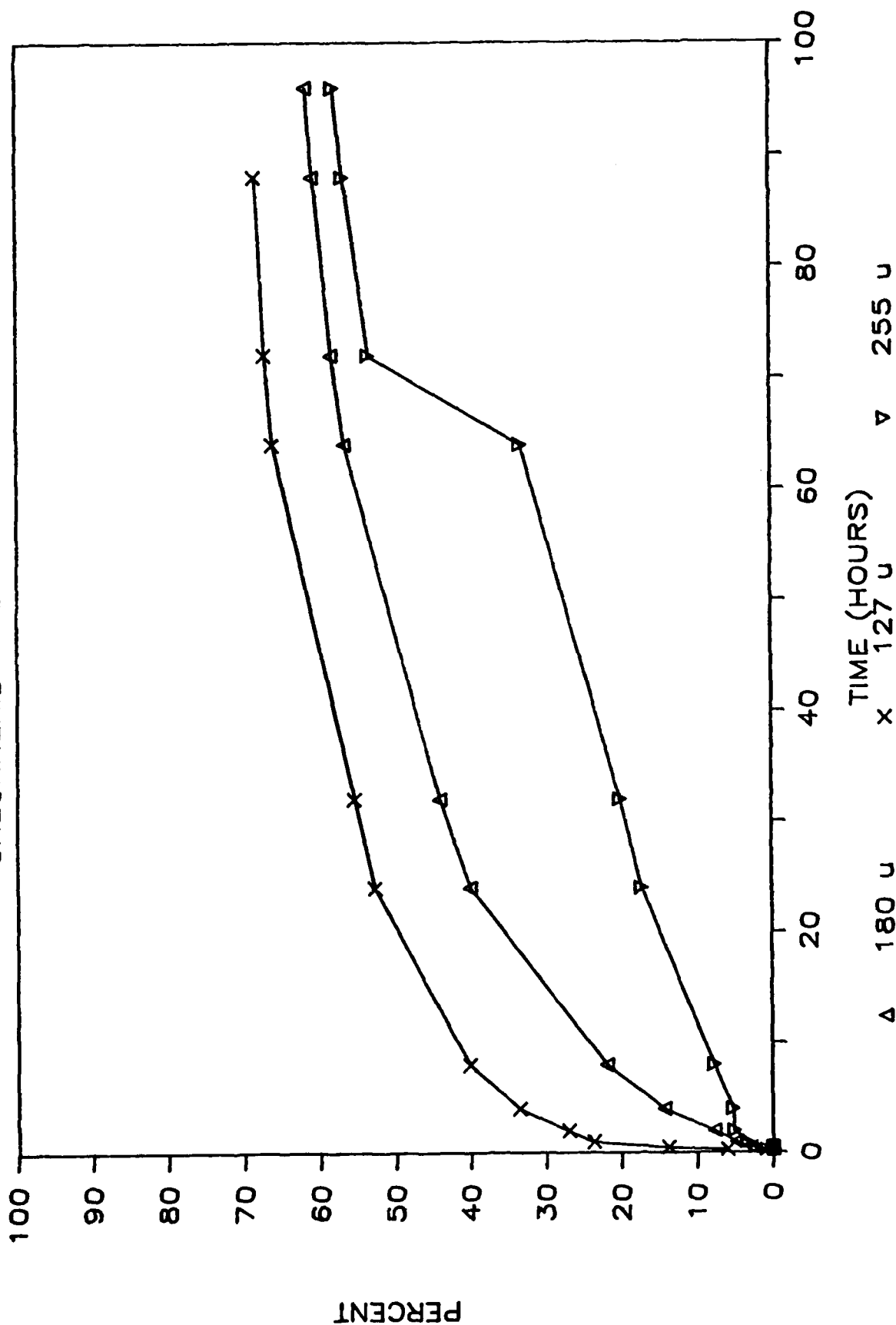


FIGURE 14

CUMULATIVE RELEASE CHLORHEXIDINE DIPHOSPHANILATE



a) Ampicillin Trihydrate Fabrics

Initial drug release measurements were made with 20% drug fabric into 40 ml of 37°C buffer solution. Ampicillin trihydrate released only about 2% in the first hour and 4% in the first day or week (Figure 15). However addition of 0.001% sodium dodecylsulfate (SDS) to the buffer increased the rate of release to 15% in the first hour and 79% in one day. Ampicillin decomposition is significant and compromises the release data at longer time periods. Incorporating the SDS in the fabric also increases the rate of release of ampicillin from a 20% ampicillin trihydrate fabric. Addition of 1% SDS in the fabric gave an almost immediate release of ampicillin (87% in one hour). Since the fabric weight was 32 mg, the amount of SDS was 0.32 mg. If this diffused into 40 ml, it would give a maximum solution concentration of only 0.0008%.

These tests were then repeated in the small reservoir cell, without agitation and at room temperature (Figure 16). In these tests about 13 mg of 20% ampicillin trihydrate fabric was placed in contact with 1 ml of buffer, SDS, or serum. Without SDS in the fabric or buffer the drug release was again very slow (1% in one hour and 8% in one day). Release of ampicillin into 0.001% SDS was faster (1% in one hour but 17% in one day). Again the addition of 1% SDS to the fabric greatly increased the rate of drug release (57% in one hour and 84% in one day).

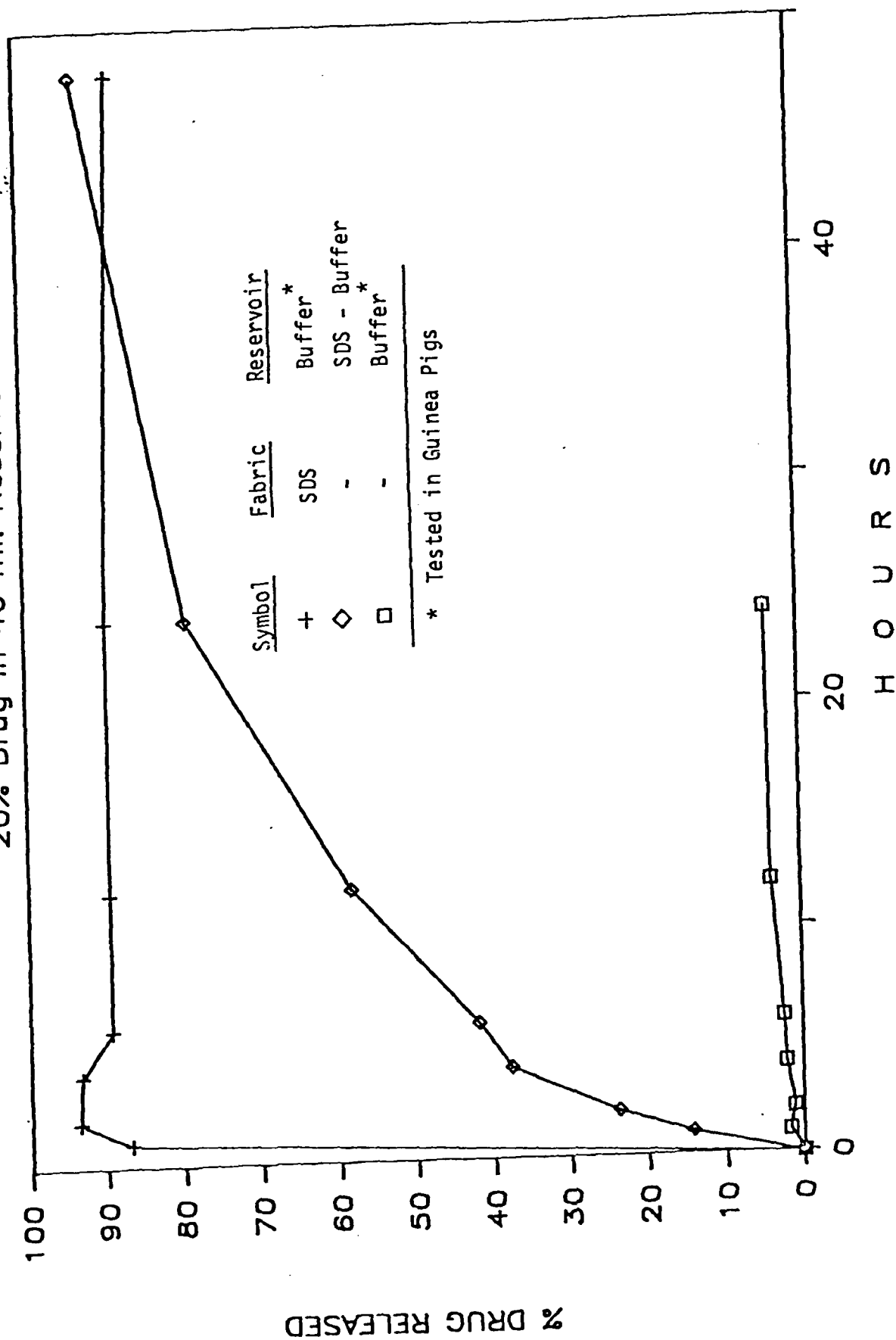
The most important aspect of drug release would be the release into a wound exudate. Serum (rabbit) was chosen as the most practical simulation of this medium for preliminary tests. Calf serum which is a pooled normal mycoplasma-tested virus-screened material has recently been obtained from GIBCO for future experiments. BIOTEK recently obtained an HPLC with ultraviolet detection capability to 214 nm. This instrumentation allows antibiotic measurement in complex systems such as serum. Appropriate methods were found for ampicillin (Tanigawara, et al, 1982) and for clindamycin (Landis, Grant, and Nelson, 1980) using HPLC with a C-18 column and uv detector.

For ampicillin a methanol/water system was suggested and, therefore, we decided to deproteinize the serum with methanol (Henry, Connor, and Winkelman, 1974). The precipitate of 8 volumes of methanol and 1 volume of serum was allowed to stand for 15 minutes prior to centrifuging. During the development of this procedure, the deproteinizing conditions were found to be important and a second peak was found for anhydrous ampicillin which was not present in the other ampicillin samples. A second peak also occurs in serum samples which have been in contact with ampicillin for several days. This is undoubtedly due to antibiotic decomposition. Solutions of pure drug can be followed in separate experiments to determine the rate of ampicillin (and clindamycin) decomposition in buffer and serum. The effect of decomposition on the uv assay of in vitro drug release can then be determined.

After the HPLC method was developed, fabric of ampicillin trihydrate (20%) was studied in the small reservoir cell using 1 ml of rabbit serum. Samples were taken at one hour intervals and after one day. These were analyzed using the HPLC conditions of Table 12. Representative chromatograms are shown in Figure 17. For the fabric without SDS the drug release (1% in one hour and 24% in one day) was faster than into buffer,

AMPICILLIN HYDRATE FABRIC RELEASE

20% Drug in 40 ml. Reservoir



INTO 1 ml RESERVOIRS

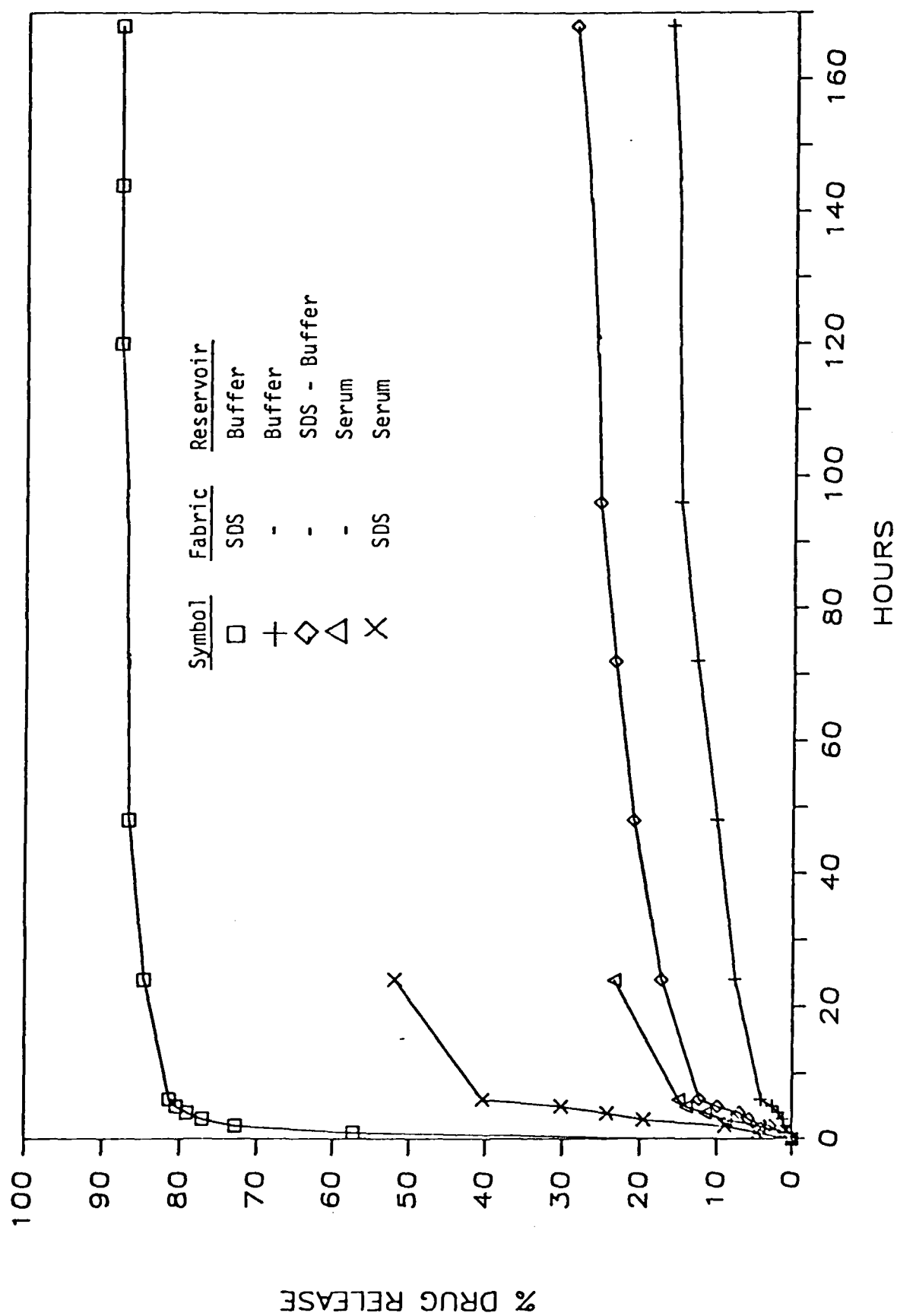


TABLE 12

CONDITIONS FOR AMPICILLIN ASSAY OF SERUM BY HPLC

Instrument Detector-Wavelength = 214 nm
 -Flow Rate = 0.6 ml/min.

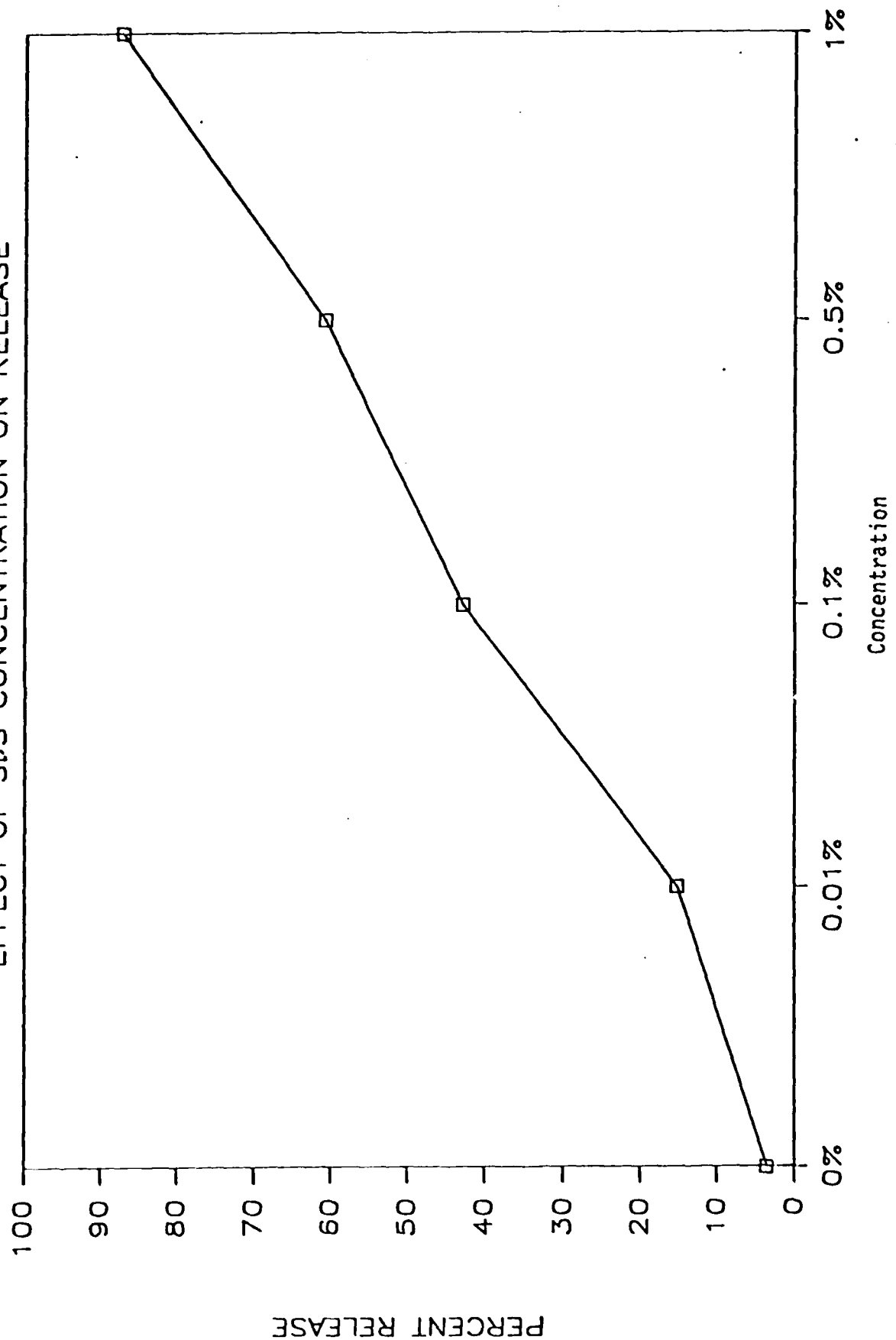
Mobile Phase - Methanol/Buffer = 30/70
 Buffer (Phosphate) = pH 7, .017M

Sample - Protein Free Filtrate = 25 μ l

Column - Radial-Pak, μ -Bondapak, C-18 (5 μ m, 8 mm x 10 cm)

Retention Time - 8 Minutes

EFFECT OF SDS CONCENTRATION ON RELEASE



dissolved in 10 ml of chloroform with mixing. Water, 10 ml, is added with vigorous mixing to dissolve the drug. With this system the mean recovery was $104 \pm 0.9\%$ (S.E.M.) for five samples.

6) Effects of S.D.S

In all of the previous studies when sodium dodecylsulfate, S.D.S., is added either to a drug-polymer matrix or to the diffusion media, the rate of drug release increased dramatically. Figure 23 demonstrates the effect of increased S.D.S. concentration in the polymer matrix on release. The data may be explained in part by either an increase in fabric wettability or an increase in drug solubility. To test the latter hypothesis, 60 mg of ampicillin trihydrate was suspended in 2 ml of distilled water with various concentrations of S.D.S. mixed for 15 minutes, and the excess separated by centrifugation. The amount of drug in solution was determined by u.v. absorption at 240 nm. The results, shown in Table 16, demonstrate a significant increase in drug solubility in the presence of S.D.S. S.D.S. appears to be an excellent material for regulating the release of ampicillin from a polymer matrix.

b. Clindamycin Materials

Clindamycin was the first material to be received and was the most difficult to analyze. Clindamycin has many structural groups which could be used as the basis of an analysis (thio, hexose, pyrrolidine, and amide groups). It was also expected to be a useful uv absorber. Unfortunately, clindamycin in acid or base did not absorb above 240 nm.

Attempts were made to develop a colorimetric assay using potassium permanganate (Draeger tube reactant for organic sulfides), an iodometric assay (USP assay for several antibiotics, including ampicillin), a hydroxylamine ferrous ammonium sulfate colorimetric assay (also USP for antibiotics), and a gas chromatographic procedure (USP for clindamycin). None of these methods were both simple and sensitive. An HPLC method was noted which used an uv detector at 214 nm; therefore we explored the lower uv range.

The gas chromatographic method described in the USP included a derivitization step with acetic anhydride. This method was tested and could be used as an alternative. An HPLC method is also available (Landis, Grant, and Nelsen, 1980). A microbiological method was submitted to us by Dr. Zurenko at Upjohn.

Clindamycin is available only as the hydrochloride and as clindamycin esters. The base form of clindamycin was prepared at BIOTEK by the neutralization of a clindamycin-HCl aqueous solution. The base was readily precipitated in water, filtered, and washed. Less soluble salts could also be easily prepared by precipitation with other anions (e.g., sulfate and phosphate).

1) Clindamycin Fabric Studies

Clindamycin-HCl does not codissolve with the polymer in methylene chloride solutions. However an excellent suspension is formed in

TABLE 15

STABILITY OF AMPICILLIN

Medium	Time (hours)	% Recovery						
		<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>24</u>	<u>48</u>	<u>96</u>
		<u>H.P.L.C. Assay</u>						
Phosphate Buffer								
pH 7.4		102	108	110	101	95	49	-
pH 7.4		98	92	-	91	86	65	-
.001% SDS								
pH 6.5		100	106	101	99	90	-	88
Calf Serum		109	100	107	96	37	0	-
Rabbit Serum		100	-	97	-	95	84	60
		<u>U.V. Assay</u>						
Phosphate Buffer								
pH 7.4		99	100	101	100	95	92	-
pH 7.4		100	102	97	100	94	-	147
.001% SDS								
pH 6.5		106	100	100	90	90	-	100
Calf Serum		162	116	85	116	37	5	-

rapidly than when tested above indicating that some of the microcapsule wall was removed during incorporation into the fabric.

This study indicates that it is feasible to prepare fabrics which incorporate microcapsules with desired release characteristics.

5) Ampicillin Stability

As previously noted ampicillin is not stable in solution, and in fact, an impurity was detected in the material supplied to us by Bristol Laboratories. Conversations with Mr. George Bittner of Bristol Laboratories confirmed this problem, but unfortunately he could offer no simple solutions. Based on their experience, we studied the stability of the drug in a number of solvent systems. Approximately 5 mg of ampicillin trihydrate was dissolved in 20 ml of solvent and at appropriate intervals 300 μ l samples were mixed with 2.4 ml of methanol. Serum samples were then centrifuged to remove precipitated protein. The extract was then analyzed by H.P.L.C. and diluted 1/5 (v/v) with methanol and analyzed by direct u.v. absorbance. For both direct u.v. and H.P.L.C. the detection wavelength was 214 nm. Samples assayed 15 minutes after preparation of the solution were considered as 100% recovery. Appropriate blanks were included.

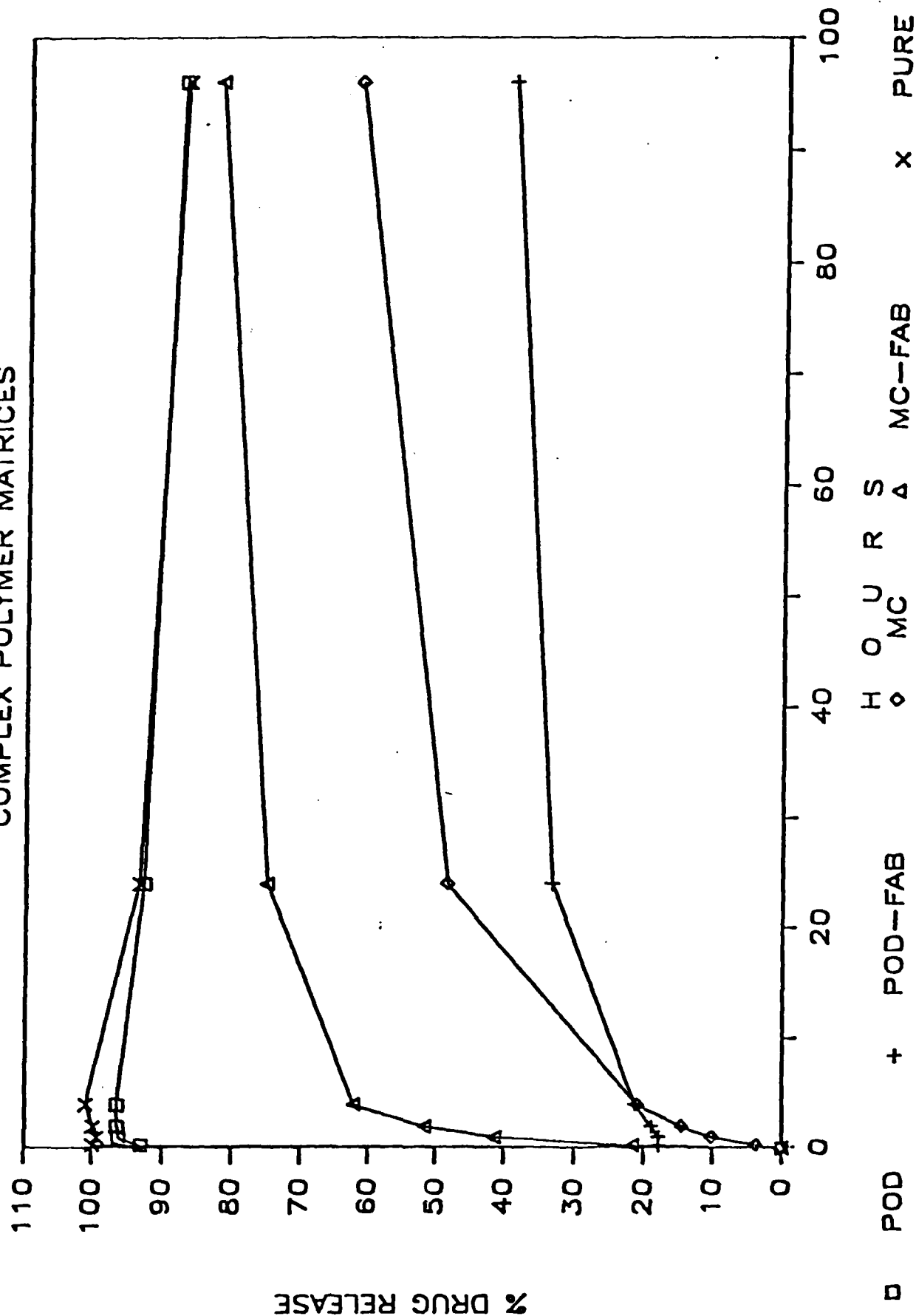
The data is shown in Table 15. The H.P.L.C. assay clearly shows that there is significant degradation of ampicillin in all solutions. In all conditions except calf serum, there is adequate stability for approximately 24 hours, but after 24 hours major losses occurred in all media except perhaps 40 mM phosphate buffer pH 6.5 which gave 88% recovery at 96 hours. The degradation in the S.D.S. containing buffer is interesting since S.D.S. increases the solubility of ampicillin (See Section III.C.3.a.6). Comparisons of the H.P.L.C. and u.v. assays done on the same samples show that with ampicillin direct u.v. assay can lead to spurious results. Ampicillin degradation apparently leads to products which also absorb in the u.v. region and significant increases in apparent recovery were observed in calf serum and in the 96 hour sample in the S.D.S. containing buffer.

These observations suggest that particularly when direct u.v. absorbance assays are used the diffusion media should be replaced at least every 24 hours if not more often.

In order to assay antibiotics in the drug-polymer matrices it is necessary to either find a solvent in which both are soluble or develop an extraction procedure. While the polymer is soluble in dioxane, chloroform, and methylene chloride, ampicillin is insoluble in these materials. Since dioxane is a carcinogen it was not studied. The addition of methanol to methylene chloride does allow solution of ampicillin. Greater solubility is achieved when either excess sodium hydroxide or a 1:1 molar ratio of sodium hydroxide to ampicillin is also included. However, in these solutions 40% or greater degradation occurred in 30 to 60 minutes. In methanol or water with a 1:1 molar ratio of sodium hydroxide and ampicillin only 12% of the drug was lost in one hour.

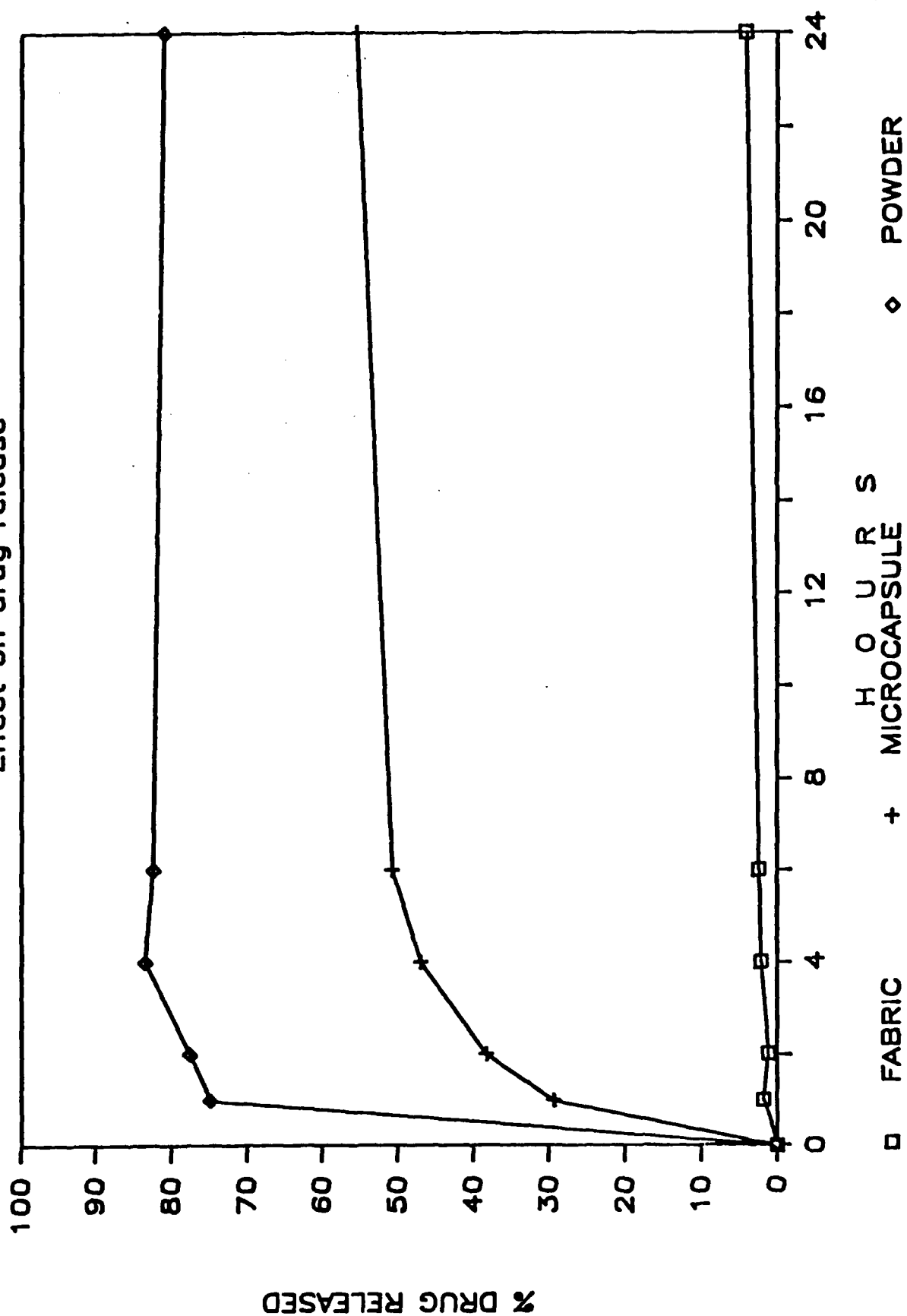
An extraction procedure based on partition of polymer into chloroform and ampicillin into water was developed. Approximately 50 mg of matrix is

DRUG RELEASE FROM COMPLEX POLYMER MATRICES



AMPICILLIN-POLYMER MATRICES

Effect on drug release



AMPICILLIN TRI. MICROCAPSULE RELEASE

Effect of Polymer Coating Level

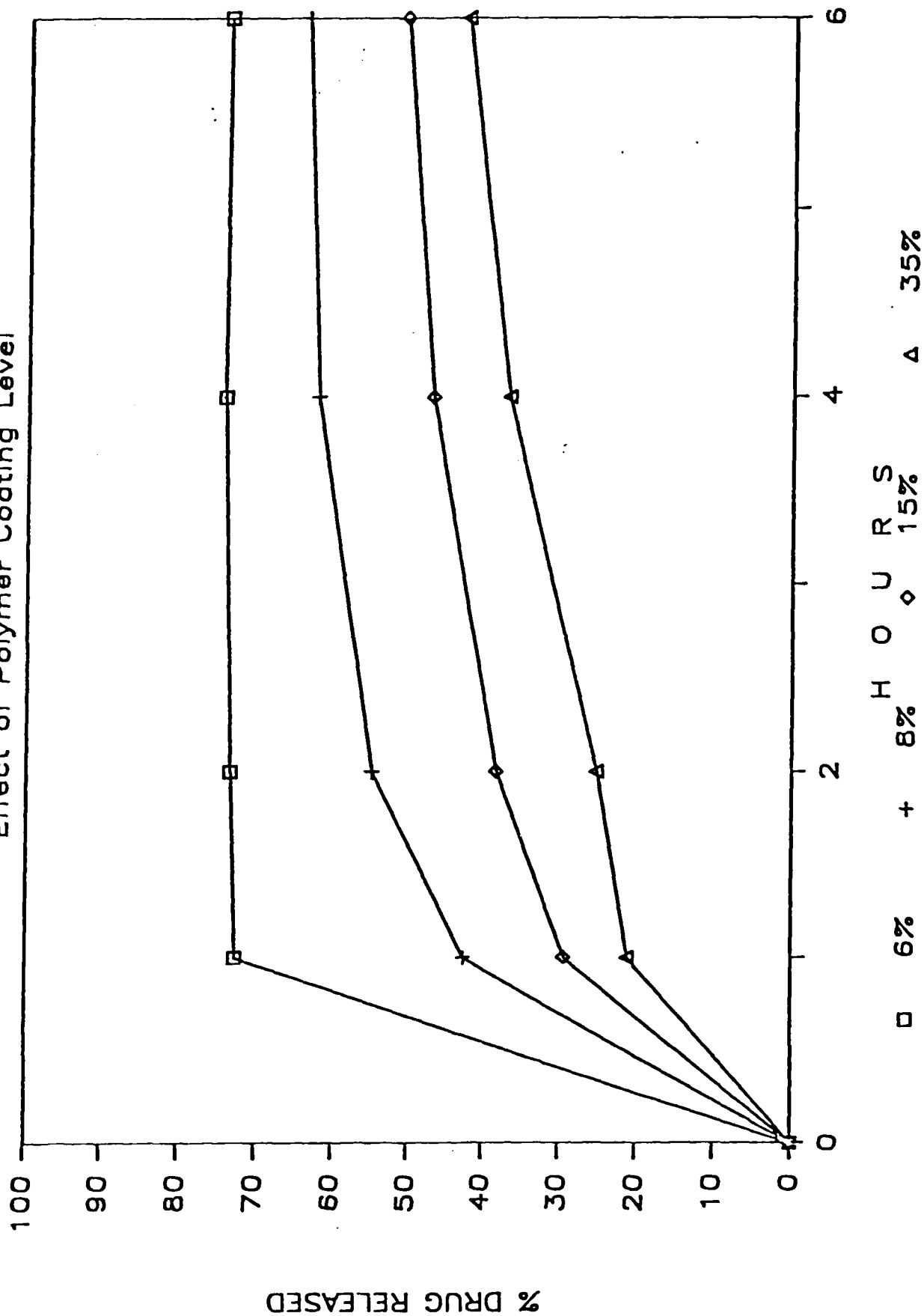


TABLE 14
CUMULATIVE % DRUG RELEASE FROM AMPICILLIN
POWDERS INTO 40 ml RESERVOIRS

<u>Sample</u>	<u>% Drug</u>	<u>Hours</u>	<u>75-106</u>	<u>212-300</u>	<u>425-600</u>
Ampicillin sodium	20	1	88	84	78
		6	88	89	83
		24	84	92	94
	5	1	122	105	95
		6	127	111	109
		24	123	108	111
		48	115	102	105
	20	1	85	75	79
		6	92	82	89
		24	86	81	85
	5	1	71	34	18
		6	79	39	23
		24	86	42	24
		48	84	42	23
Ampicillin anhydrous	20	1	89	84	60
		6	88	90	71
		24	84	85	71
		6 day	64	64	56
	5	1	82	54	36
		6	80	55	38
		24	71	47	29
		6 day	51	40	25

the problems of ampicillin decomposition, the latter preparation method is preferred.

Because of the fast release from the 20% ampicillin powders, at three chosen sieve ranges, powders were prepared at 5% of each of these drugs. The drug release was studied in the standard (40 ml) cells with buffer. Data was taken at several times and for several days. However, a summary of the data, as shown in Table 14, is sufficient to show the trend. In all cases drug decomposition occurs concomitantly with drug release. We see no promising materials in this test, although drug release in a small cell and *in vivo* might be slower. Lower drug loading did not significantly decrease the rate of ampicillin release. Microencapsulation appears to offer a better alternative for the preparation of ampicillin particles.

3) Ampicillin Microcapsules

Recently microcapsules were prepared using sodium ampicillin and ampicillin trihydrate. The first encapsulation used nondescript core of sodium ampicillin and should be repeated. This was the first drug encapsulation using the small air suspension coater. The second encapsulation used ampicillin trihydrate (H. Reisman) in the 2-inch coating chamber. Approximately 14 grams of core material was coated to 35% polymer wall with a 2% polylactide solution. Small samples were taken at 6, 8, and 15% coating.

Drug release studies were performed on unsieved material of the smallest coating samples (6 and 15% coated). At 8 and 35% coating, sieve fractions of 210-300 μ m were used for the drug release experiment. The data are shown in Figure 20. Data at more than 6 hours was compromised by drug decomposition. However in this preliminary study the slow release of ampicillin was demonstrated, and the effect of higher coating levels giving slower drug release is evident.

4) Complex Ampicillin-Polymer Matrices

A comparison of the *in vitro* release rates of the three formulations of ampicillin trihydrate is shown in Figure 21. From this data it appeared that better control of release rate could be achieved by combining non-woven fabrics with either powders or microcapsules. A preliminary experiment was conducted to test the hypothesis that powders and microcapsules could be incorporated into the fabric. Microcapsules 100 - 600 microns with 65% ampicillin trihydrate or ampicillin powders 80% drug 100 - 600 microns were suspended in a Wurster air suspension coating chamber. The coating nozzle was replaced with a fabric preparation nozzle and 10% polylactide in methylene chloride was blown through the suspended particles. As the fabric strands dried they trapped the particles and the resultant fabric was collected on a gauze backing.

Drug release from these complex matrices is shown in Figure 22. Pure drug and the powder both released almost instantly (<15 minutes), and microcapsules released much slower. Powder release was significantly slowed by incorporation into the fabric. On the other hand the microcapsules incorporated into polymer fabric release ampicillin somewhat more

TABLE 13

SIZE DISTRIBUTION OF AMPICILLIN POWDERS

(Data is % of Total Milled Sample)

Size (μ m)	Ampicillin Trihydrate		Ampicillin Sodium		Ampicillin Anhydrous	
	5%	20%	5%	20%	5%	20%
> 600	3.0	2.9	9.8	6.8	3.2	9.1
600-425	19.7	20.6	19.5	15.8	15.3	15.1
425-300	39.1	44.7	28.1	25.2	37.8	23.8
300-212	19.6	22.4	21.1	22.8	23.5	22.7
212-150	7.9	7.4	11.4	15.0	7.4	11.8
150-106	5.6	2.4	7.0	9.8	7.1	10.8
106-74	2.8	0.6	2.2	4.0	3.5	4.4
74-35	1.0	0.1	0.7	2.0	1.8	2.0
< 38	0	0	0.1	0	0.2	0.3
Yield	85	57	94	80	89	90

FIGURE 19

AMPICILLIN TRIHYDRATE/SDS FABRIC

IN VITRO RELEASE (40ml)

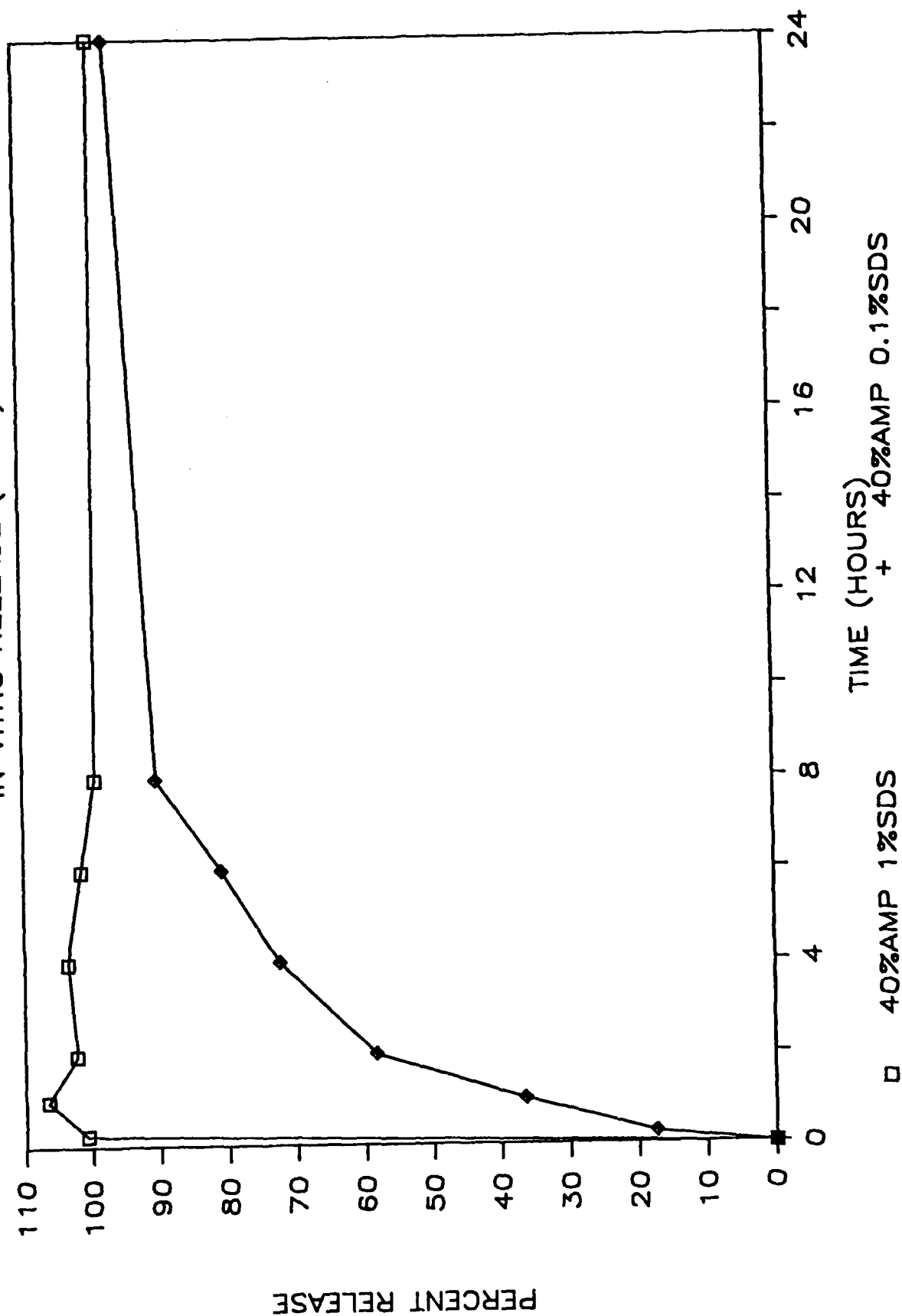
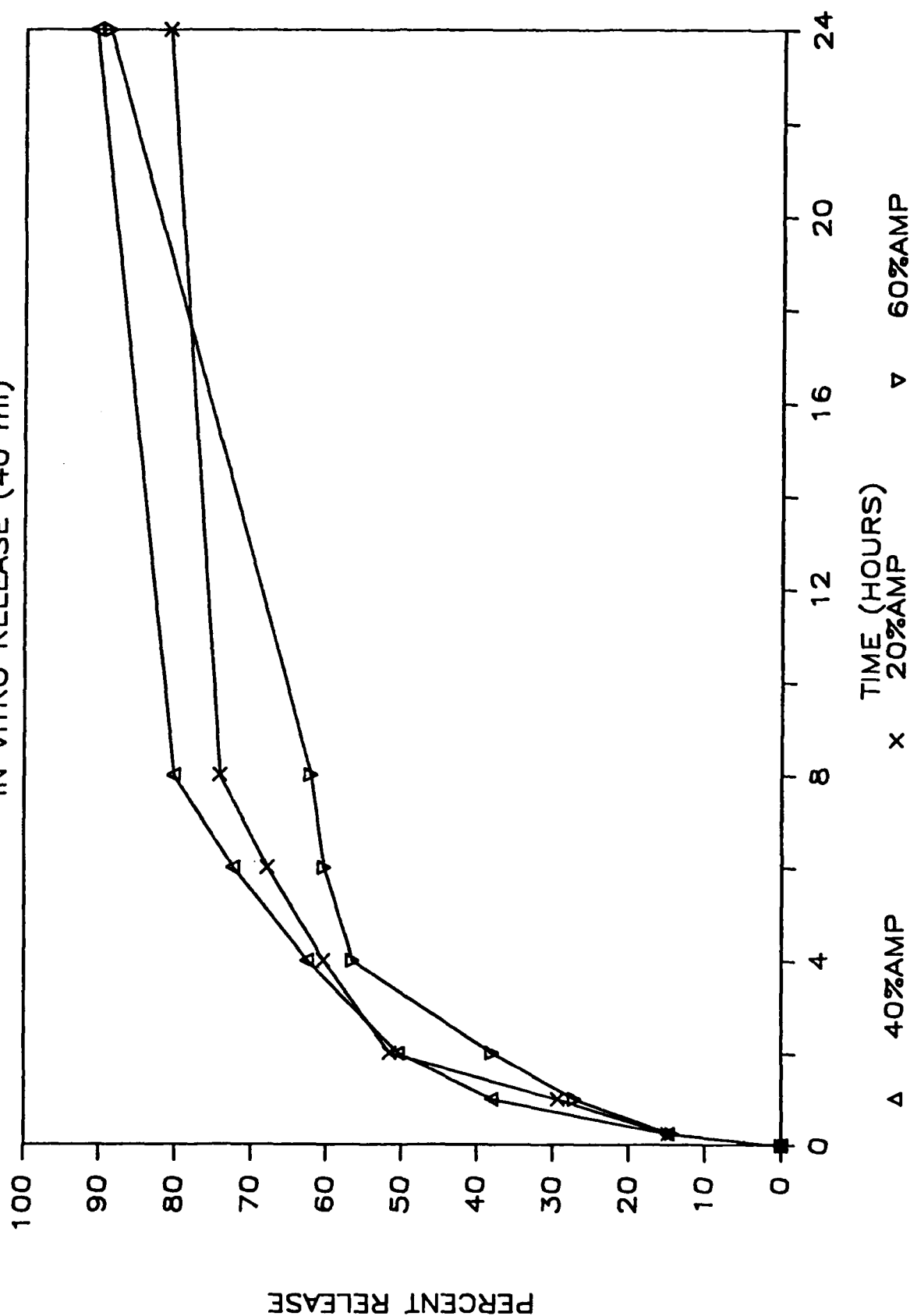


FIGURE 18

AMPICILLIN TRIHYDRATE FABRIC

IN VITRO RELEASE (40 ml)



but slower than into 0.001% SDS. For the fabric containing 1% SDS the drug release (5% in one hour and 52% in one day) was faster than without SDS in the fabric, but it was slower than the release of drug from this SDS fabric into buffer.

Increasing the concentration of ampicillin trihydrate in the fabric had relatively little effect on the release profile (Figure 18). The difference observed in percent drug release are slight and insignificant. Therefore increasing drug loading of the fabric will provide higher doses but not alter the time course. As shown previously for 20% drug loading the addition of 1% SDS to a 40% ampicillin trihydrate fabric gave virtually immediate release (< 15 min) (Figure 19). However, the addition of 0.1% SDS had little effect.

b) Anhydrous Ampicillin Fabrics

The measure of ampicillin release from the anhydrous ampicillin fabric was measured at 240 nm without regard to the presence of the impurity found by the HPLC method. Only a 20% anhydrous ampicillin fabric was prepared.

In the standard 40 ml cell the drug release into buffer was slow but significant (5% in one hour and 15% in one day). It was increased by addition of 0.001% SDS to the buffer (13% in one hour and 76% in one day). This data was similar to that for ampicillin trihydrate. In the small 1 ml cell the drug release was slower than in the agitated large volume cell at 37°C. In the small cell the fabric released 2% into buffer in one hour and 12% in one day. With SDS in the buffer the rate of release was still slow (1% in one hour and 14% in one day).

c) Sodium Ampicillin Fabrics

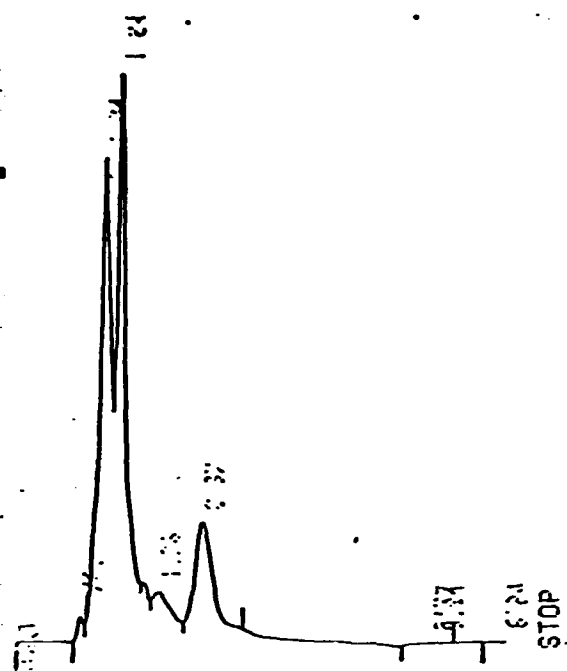
Sodium ampicillin did not form a fluffy non-woven fabric. The powdery material released 62% of the ampicillin in one hour and 74% in one day in the standard 40 ml cell using buffer. In the 1-ml cell the release was almost as fast (42% in one hour and 67% in one day). Measurements were not made in the presence of SDS.

2) Ampicillin Powders

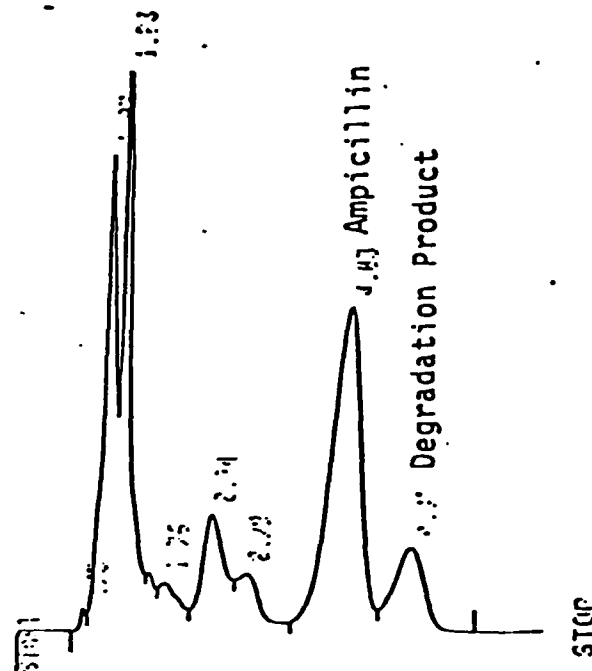
For the first series of powders, films were prepared with 20% ampicillin trihydrate and sodium ampicillin. These films were cut and the pieces fed through the hammer mill. However, in this experiment dry ice was not mixed with the film and passed through the grinder as in the previous experiments. This avoided the build up of moisture on the product due to moisture condensation as the dry ice sublimed. The absence of dry ice did not appear to affect the product yield or the size distribution of the powder. This data is shown in Table 13.

A second series of powders were prepared with 5% and 20% anhydrous ampicillin, and 5% of sodium ampicillin, and ampicillin trihydrate. These powders were prepared with dry ice, but the product was quickly transferred to a vacuum oven and the dry ice sublimed into a vacuum line. A dry powder was obtained with yields as described in Table 13. Based on

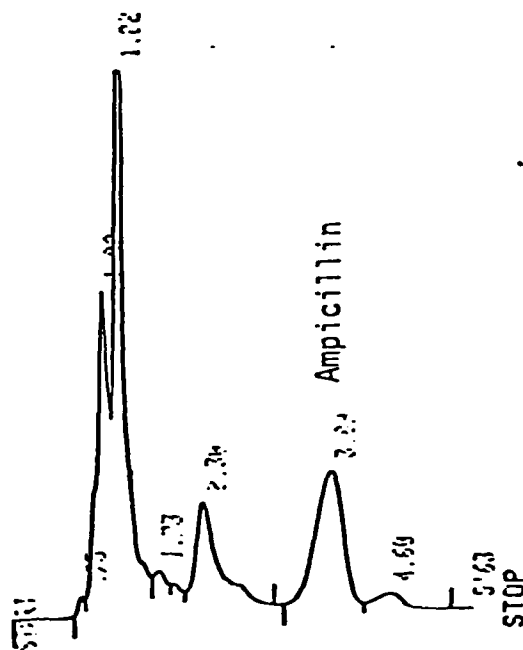
FIGURE 17

AMPICILLIN ANALYSIS BY HPLC

Serum. Blank



Neat Ampicillin and Serum



Ampicillin Fabric and Serum

TABLE 16

EFFECT OF S.D.S. ON AMPICILLIN SOLUBILITY

S.D.S. Concentration (%)	Ampicillin Solubility (mg/ml)
0.000	6.31
0.001	6.30
0.01	6.21
0.1	6.22
1.0	8.98
10.0	> 20.47

methylene chloride which can be sprayed through an atomizing nozzle. A 20% drug fabric was prepared using our standard conditions.

Using 20% clindamycin-HCl fabric in the standard cell almost all of the drug was quickly released into the standard phosphate buffer (70% in one hour and 80% in one day). Fabrics were, therefore, prepared with 15, 10, and 5% drug. However, the release was not significantly slower at these higher polymer concentrations. There would be no apparent advantage to testing these fabrics in the presence of SDS. Clindamycin was released slower into the non-agitated 1 ml of buffer. The 20% clindamycin fabric released 33% of the drug in one hour and 46% in one day in the 1-ml test system.

The reproducibility of drug release from fabrics is much less than for similar powders or microcapsule materials. Also for clindamycin we have not yet developed an assay procedure for the fabric. Hence the data is based on the weight of drug added to the polymer solution for the preparation of the fabric. However we believe that the non-reproducibility is due to the drug release test method, possibly a non-reproducible wetting of the test fabric in the test cell.

2) Clindamycin Powder Studies

Powders were prepared at 20% and 5% clindamycin-HCl. The drug release was studied in the standard (40 ml) cells with buffer. Data was taken at several times and for several days. However, a summary of the data, as shown in Table 17, is sufficient to show the trend. For clindamycin, we suspect that there was drug segregation and that the small powder contains most of the drug. In all cases drug decomposition occurs concomitantly with drug release. However, we see no promising materials in this test. Powders of clindamycin base or less soluble salts, or clindamycin-HCl microencapsulation appear to offer better alternatives for clindamycin particulates.

3) Clindamycin Microcapsule Studies

Core particles of clindamycin hydrochloride were coated using the 2-inch coating chamber. Approximately 15 grams of material was coated to a level of 20%. A small sample was also taken at the 10% coating level.

The size distribution of clindamycin microcapsules at the 10% coating level determined by sieve analysis (Table 18). As with the chlorhexidine diphosphanilate microcapsules, most of the material, more than 50%, was larger than 425u. These microcapsules released nearly 100% of the drug in 15 minutes. This is probably a result of the low coating level.

TABLE 17

CUMULATIVE % DRUG RELEASE FROM CLINDAMYCIN
POWDERS INTO 40 ml RESERVOIRS

<u>Sample</u>	<u>% Drug</u>	<u>Hours</u>	<u>75-106</u>	<u>212-300</u>	<u>425-600</u>
Clindamycin-HCl	20	1	133	62	40
		6	135	64	48
		24	142	65	51
	5	1	82	49	41
		6	79	43	41
		24	51	18	14
		6 day	37	5	6

TABLE 18
SIZE DISTRIBUTION OF CLINDAMYCIN MICROCAPSULES

<u>Microcapsule Size</u>	<u>% Weight</u>
Fines	0.8%
74-105 μm	1.8%
105-149 μm	5.4%
149-210 μm	9.8%
210-300 μm	11.7%
300-425 μm	16.6%
425-600 μm	26.0%
>600 μm	27.9%

D. Physical Characterization

Physical characterization is important for understanding and improving the drug release characteristics of the powders, fabrics, and microcapsules. It is also important in developing materials which have better physical properties as wound dressings (absorptivity, improved powder flow, etc.). Finally, physical characterization is important for quality control purposes.

Size analysis by sieving in the Sonic Sifter™ is the most important physical characteristic of the powders and microcapsules. Light microscopy is used to determine particle shape and agglomeration. Scanning electron microscopy (SEM) is used to determine surface texture. An energy dispersive analysis of x-rays (EDAX) which is used in conjunction with the SEM gives information on the exposed drug on the surface of the wound dressing materials. Analysis is made of the relative quantity of a heavy element (Cl, I, S) relative to the EDAX spectrum of the pure drug.

1. Electron Microscopy

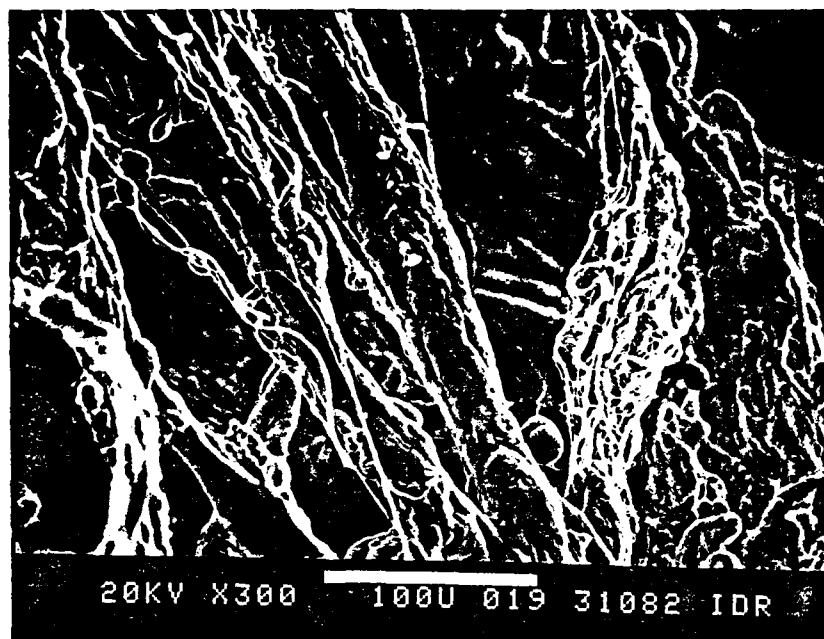
Scanning electron micrographs have been obtained for many of these samples, courtesy of Colonels Tsaknis and Mader at USAIDR. In Figure 24 is shown the povidone iodine fabrics at 20 and 50% drug loading. Excellent fabric can be formed with 20% povidone iodine, but the 50% material is much less fibrous. This is also evident from the scanning electron micrograph. The 40% drug fabric is intermediate in fibrous structure.

EDAX analysis of povidone-iodine fabric of 20 and 50% drug showed less iodine than expected. Nodules and spheres in the 50% fabric gave values of 25% surface iodine, and a thin fiber contained only 17% surface iodine. The 20% fabric sample yielded 16% surface iodine in fiber areas and about 12% in nodular areas. Perhaps surface iodine is lost in the process of SEM sample preparation and analysis. However, the analysis does not show a clear trend which can be correlated with in vitro drug release data.

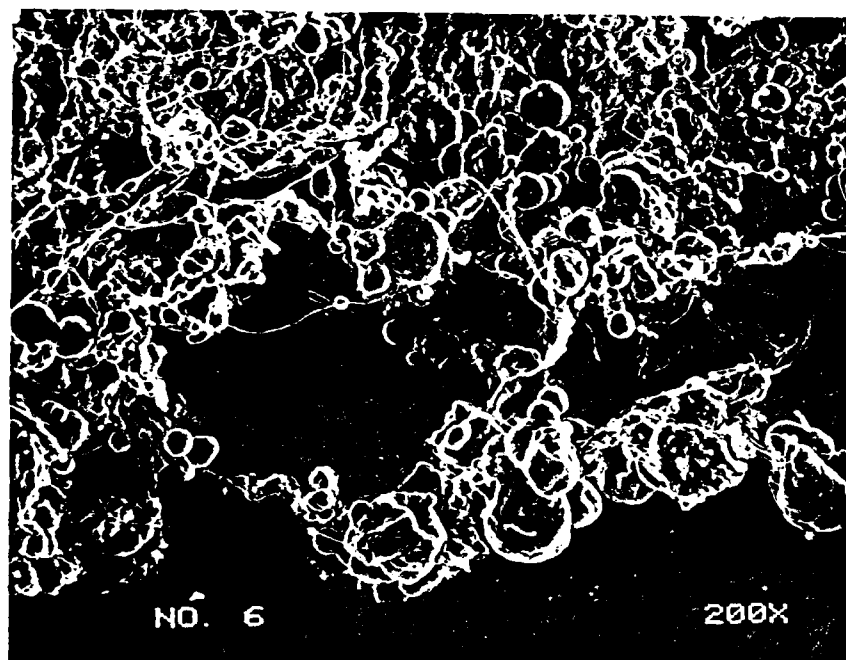
SEMs of povidone iodine powder is shown in Figure 25. This analysis shows significant segregation of iodine within the sample (20% BASF 17/12, 212-300 microns), with about 50 counts/second at the iodine peak of a round particle that may be pure povidone iodine. Background levels (2 counts/sec) were observed in the less structured area of the sample.

The surface of microcapsules is smoother than that of powders and there is less exposed drug. The amount of exposed drug has been shown to correlate well with the amount of drug release in the first hour (Contract DAMD17-81-C-1195, etidocaine-HCl microcapsules). For the povidone iodine microcapsules (212-600 microns, 30% coating), the iodine peaks were compared on pure povidone iodine, microcapsule core particles, and microcapsules coated with various quantities of polylactide (Table 19). Figure 26 shows an optical photomicrograph of these microcapsules on Tegaderm™.

Figures 27 to 29 are SEM's of ampicillin trihydrate fabrics. These non-woven fabrics are composed of intertwined strands and beads of drug/polymer matrix. When compared to control fabric (Figure 30) which

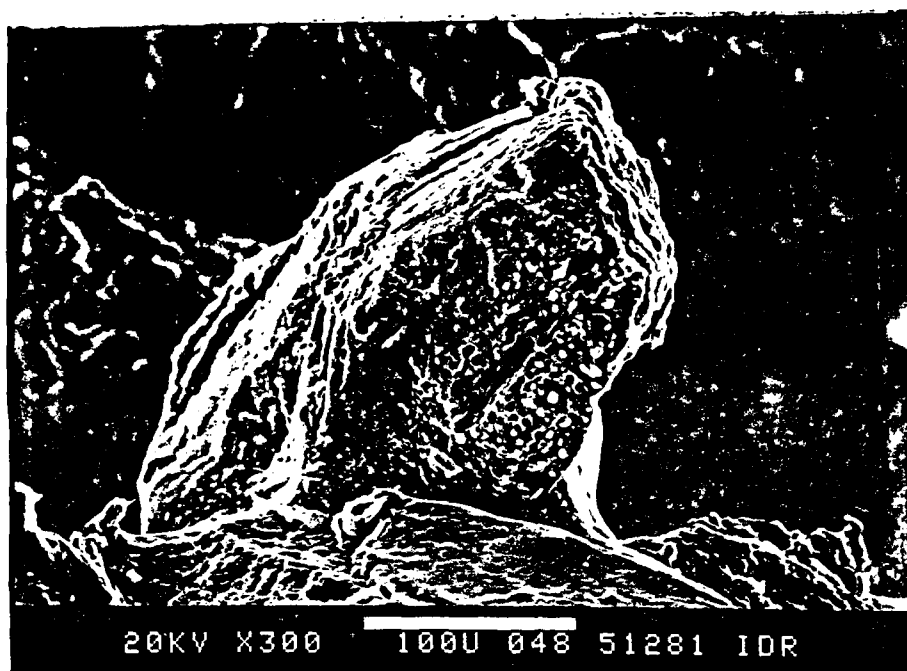


20% PVP·I₂

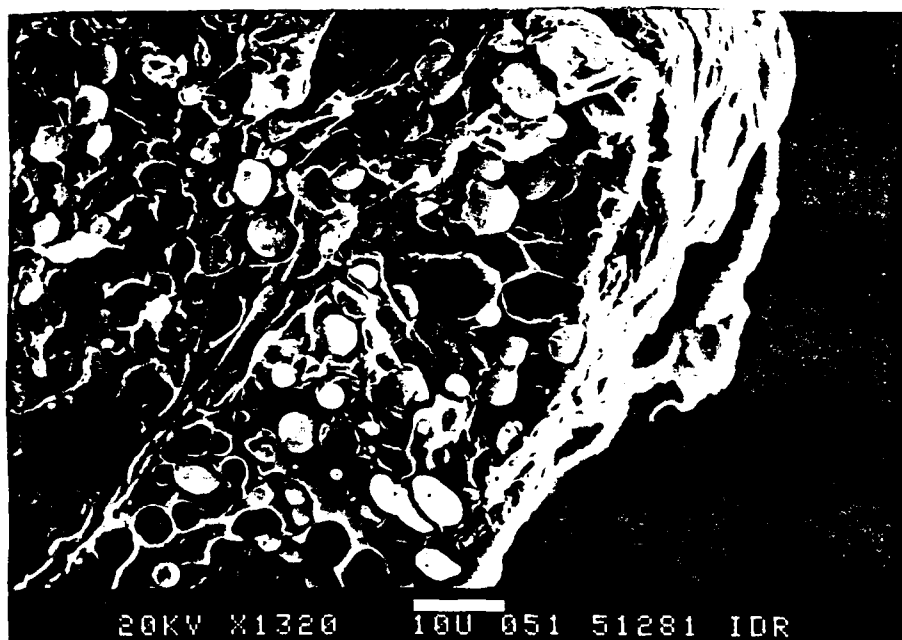


50% PVP·I₂

Figure 24 SEMs of PVP·I₂-polylactide Fabric Showing Sturctural Difference as Drug Concentration is Increased



x300



x1320

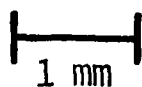
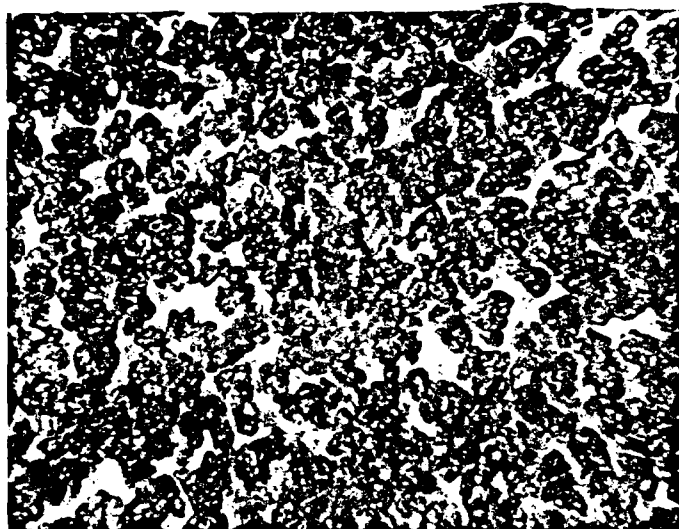
Figure 25 SEMs of PVP-I₂-Polylactide Powder of 20% Drug (212-300 microns) Showing Structure of Fractured Surface

TABLE 19

EDAX ANALYSIS OF DRUG SURFACECONCENTRATION OF PVP-I₂MICROCAPSULES

<u>Material</u>	<u>% Exposed Drug</u>
PVP-I ₂ - neat	(100)
PVP-I ₂ - core	104
10% coated M.C. (212-300 μm)	13
20% coated M.C. (212-300 μm)	3
30% coated M.C. (212-300 μm)	3

FIGURE 26



Povidone Iodine 70% In Microcapsules
(30% Poly-L(-)lactide Coating)
On Commercial Adhesive Wound Dressing

FIGURE 27

SEM OF 20% AMPICILLIN TRIHYDRATE NON-WOVEN FABRIC



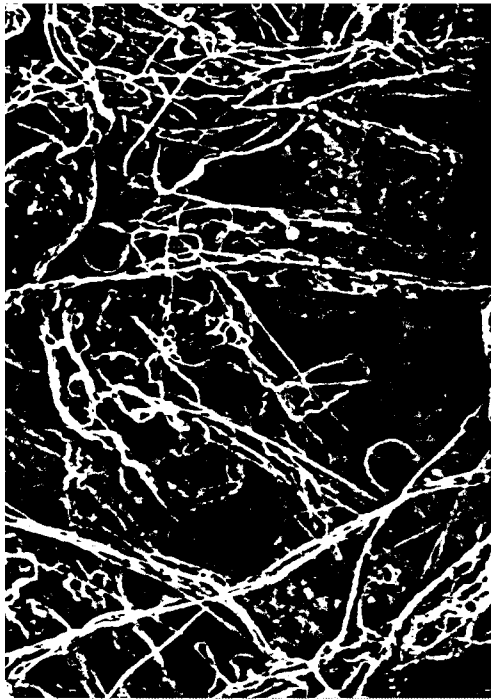
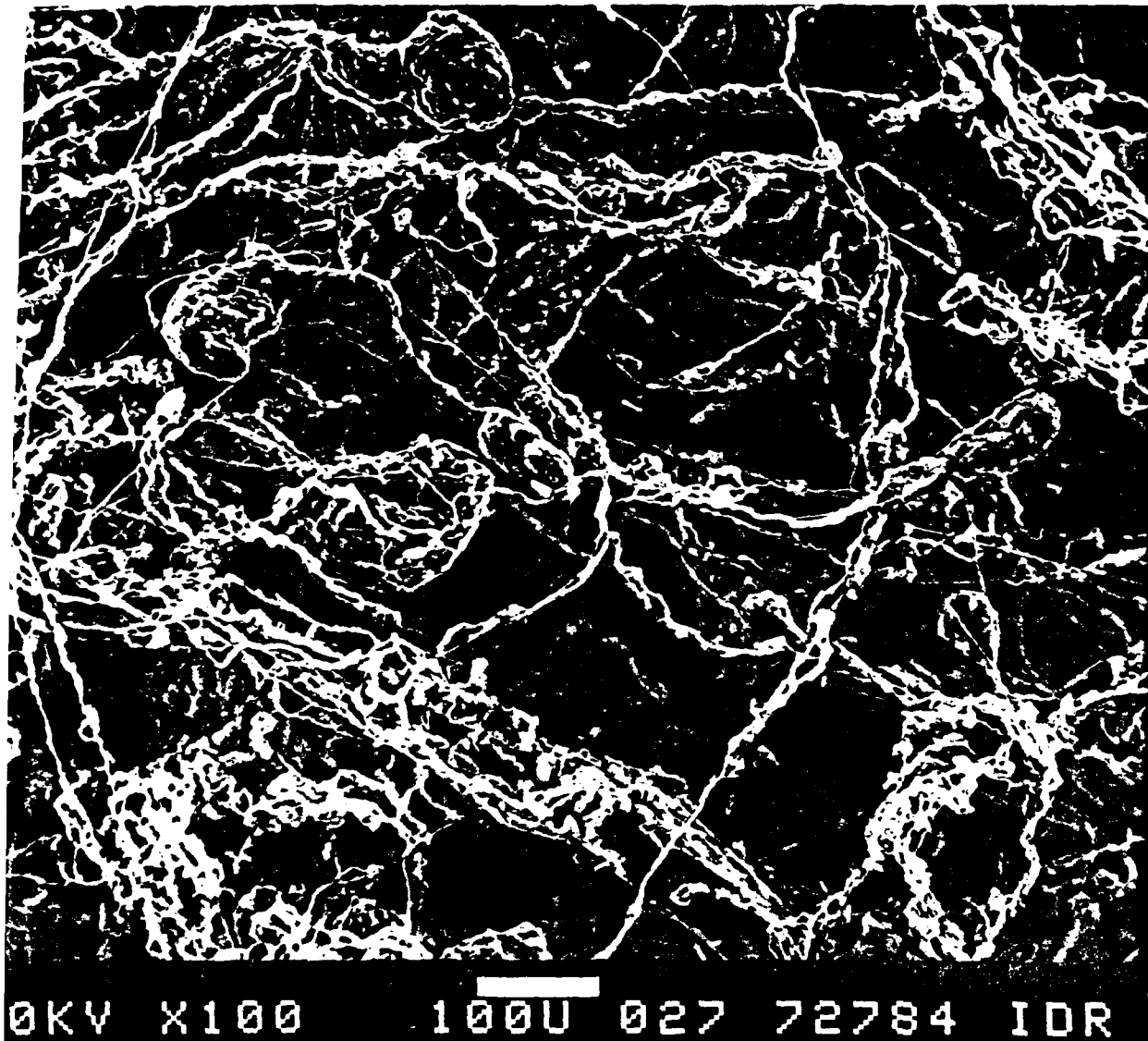
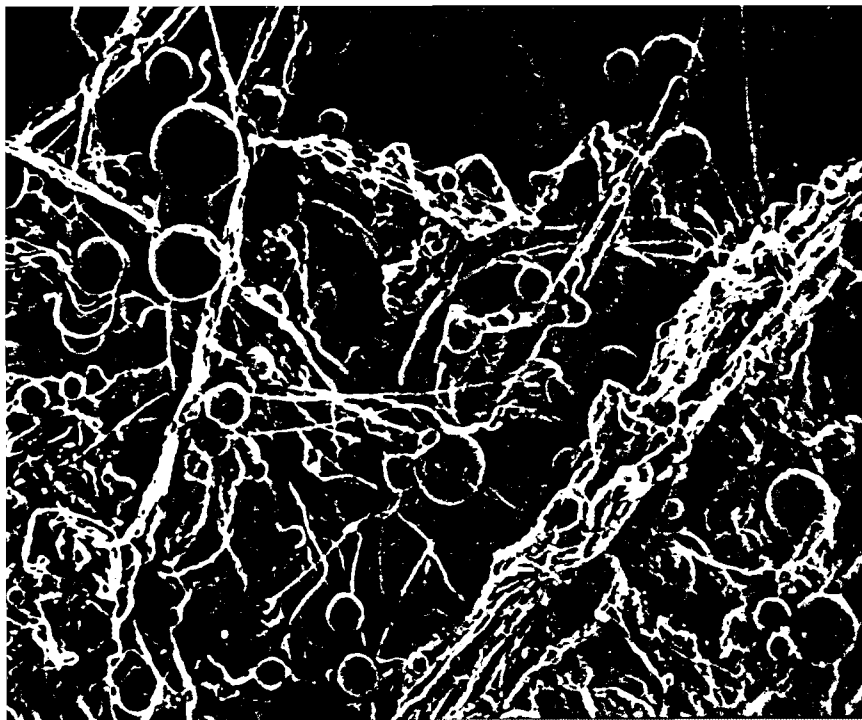


FIGURE 29

AMPICILLIN TRIHYDRATE FABRIC
60% DRUG LOAD





tains no drug the major difference is in the appearance of the bead form during fabric preparation. Pure polymer beads are smoother and spherical while the ampicillin/polymer beads are more irregular and amorphous in appearance. The higher the drug concentration (compare Figure 28, 20% drug, to Figure 29, 60% drug) the more irregular the beads. The addition of SDS, either 0.1 or 1.0 percent, to the fabrics has no effect on the morphology (Figure 31).

In order to improve the release characteristics of non-woven fabrics microcapsules and powders were incorporated to form complex matrices. Figures 32 and 33 show that ampicillin trihydrate microcapsules incorporated into a non-woven polymer fabric maintain their integrity. Morphologically this complex matrix looks more like the control fabric. The microcapsules are generally smaller and more uniform than the beads found in control fabric (Figures 30 and 31). The presence of the microcapsules seems to inhibit the formation of the larger beads. The microcapsules are much more uniform than the irregular, amorphous beads seen in the fabric with higher drug loading (compare Figure 29).

SEM's of ampicillin trihydrate, clindamycin-HCl, and chlorhexidine dihydrophosphate microcapsules prepared in the small coating chamber developed by BIOTEK are shown in Figures 34, 35, and 36. These microcapsules are less uniform than those produced in the large coating chamber. They appear to be composed of agglomerates (212 to 300u) of small microcapsules.

2. Porosimetry Measurements

On similar projects, mercury porosimetry has been found to yield useful information on microcapsule morphology. A single test gives information on (1) bulk density, by a repeatable method on a small sample, (2) interparticle void volume, (3) pore volume, with volume associated with various restricted pore radii, and (4) skeletal density which defines solid pore volumes assuming that the absolute density of the material is known. We sent samples of powders and fabrics to Micromeritics, Inc. who performed porosimetry testing on a purchase order basis.

The bulk volume of the fabrics are dependent on the method of measurement, since the material is extremely compressible. Using vernier calipers and no compression the fabric volume was $3.32 \text{ cm}^3/\text{gm}$. After filling the porosimeter tube with mercury at atmospheric pressure the volume was $1.28 \text{ cm}^3/\text{gm}$. In general, volumes larger than 123 microns in diameter are filled by the porosimeter prior to the application of pressure.

The data on the equivalent volumes for particles and fabrics of 15% procaine-HCl are shown in Table 20. For a bed of particles of 300-425 microns sieve size we assume that volumes with equivalent pore sizes of less than 10 microns are interparticle voids. Constrictions of less than 10 microns are considered pore volumes. For non-woven fabric matrices concept must change to the filtration effectiveness of a bed filter. The data indicate relatively smooth surfaces for all of the non-woven fabrics.

AD-A154 270

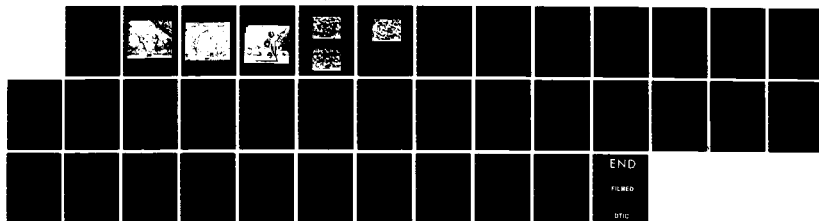
RESEARCH AND DEVELOPMENT OF WOUND DRESSING IN
MAXILLOFACIAL TRAUMA(U) BIOTEK INC WOBURN MA
M H GAY ET AL. 16 NOV 84 2110-12 DAMD17-81-C-1204

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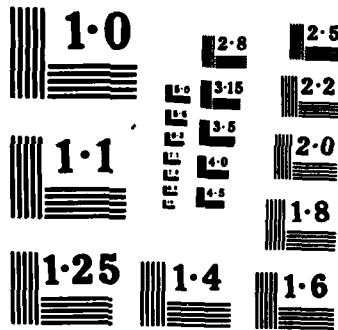
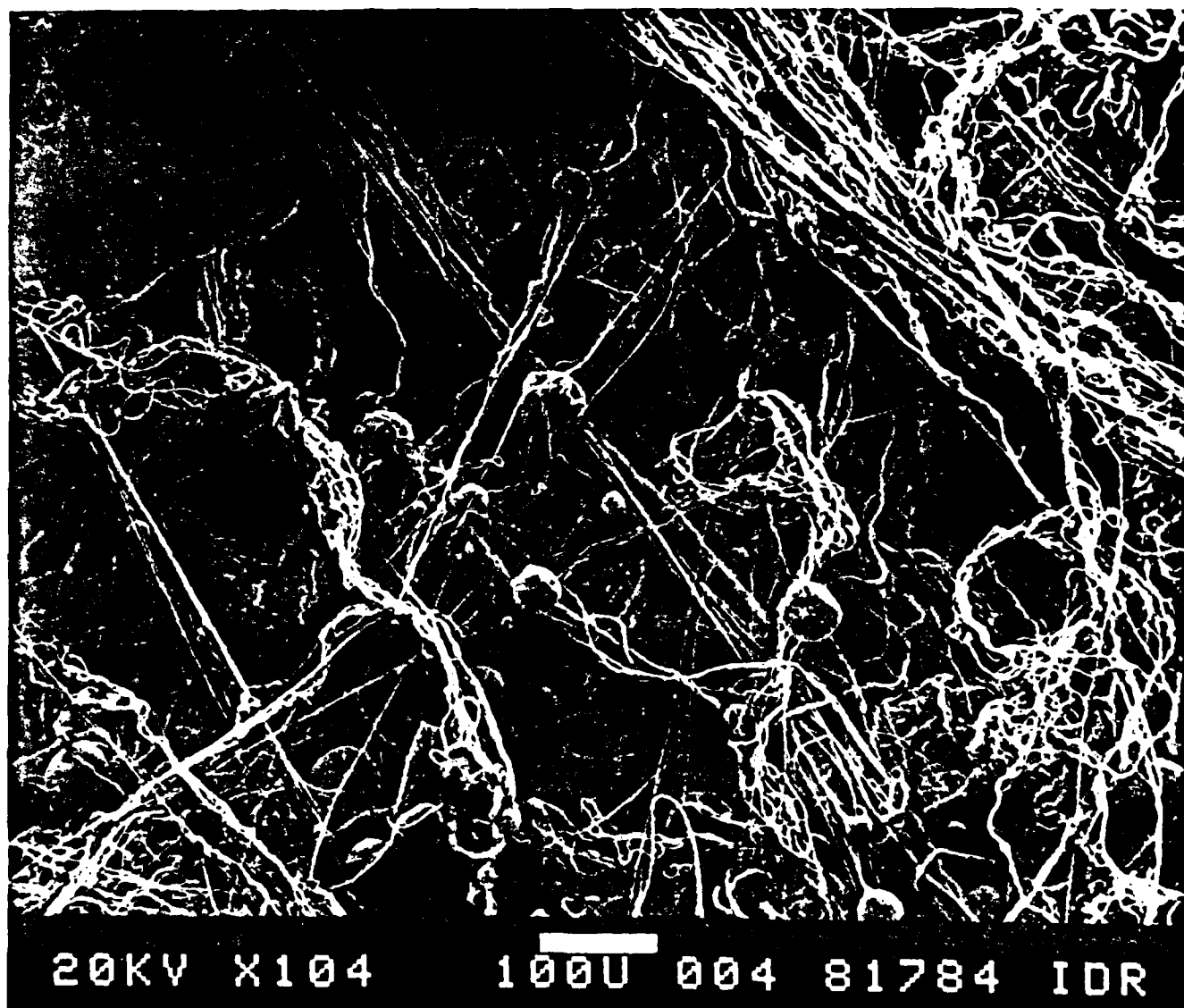


FIGURE 31

POLYLACTIDE CONTROL FABRIC
1% SDS



AMPICILLIN COMPLEX MATRIX
FABRIC/MICROCAPSULES
19% DRUG LOAD
(X100)

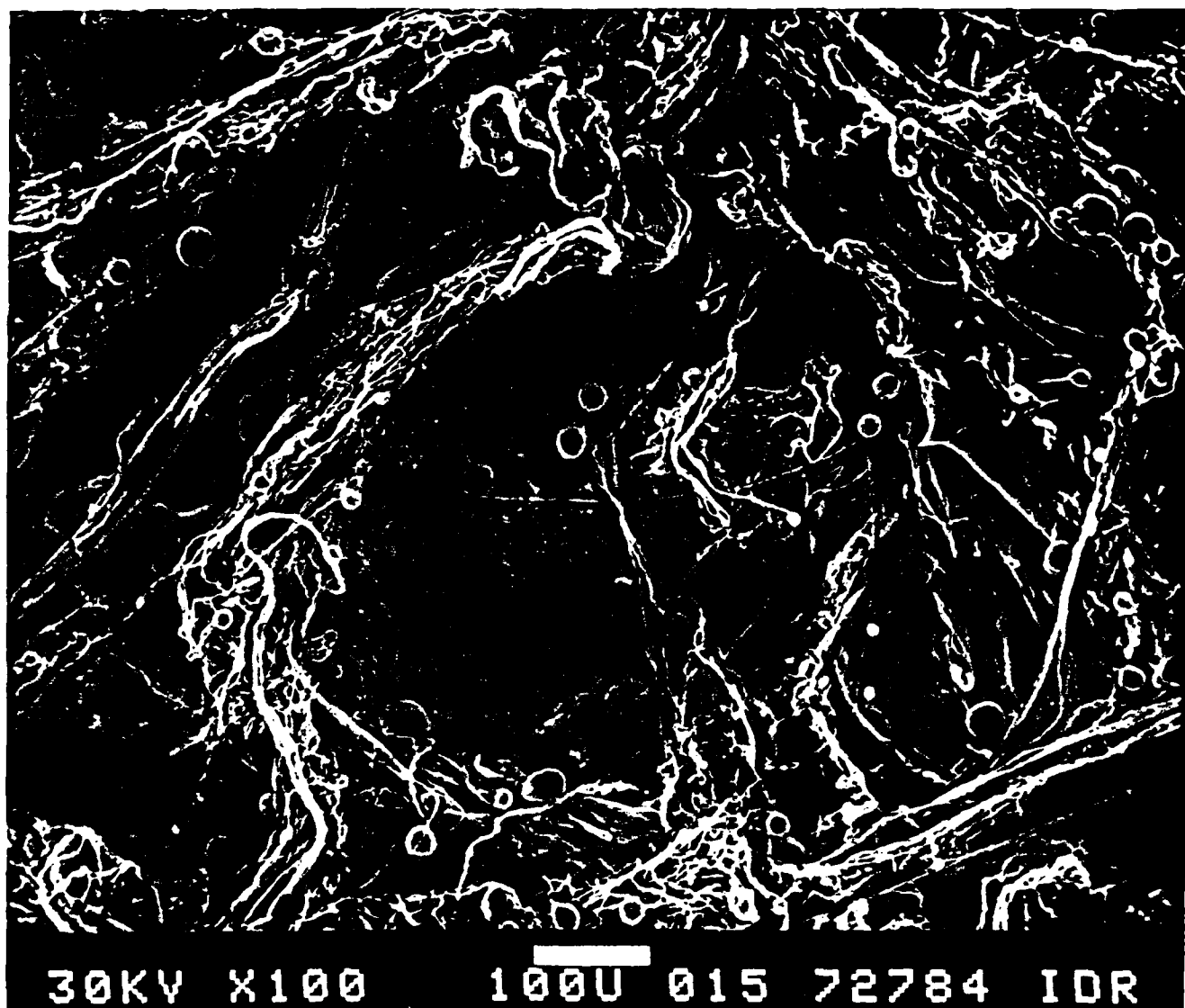


FIGURE 33

AMPICILLIN COMPLEX MATRIX
FABRIC/MICROCAPSULES
19% DRUG LOAD
(X504)



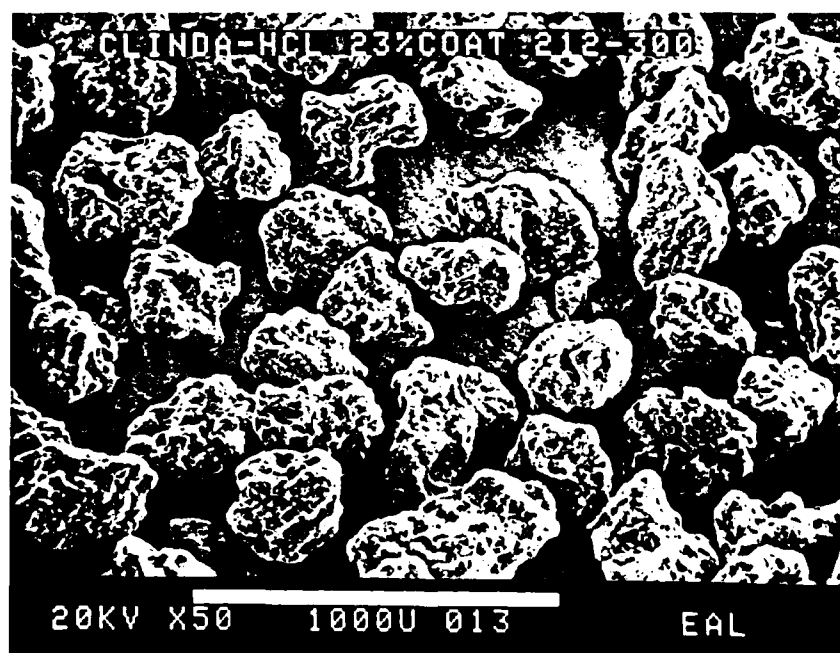
FIGURE 34 AMPICILLIN TRIHYDRATE MICROCAPSULESFIGURE 35 CLINDAMYCIN-HCL MICROCAPSULES

FIGURE 36 CHLORHEXIDINE DIPHOSPHANILATE MICROCAPSULES



TABLE 20

MERCURY POROSIMETRY VOLUMES OF
ETIDOCAINE HCl POWDERS AND FABRICS

	15% Etidocaine-HCl 300-425 μ m Powder Particles <hr/> cm ³ /gm	15% Etidocaine-HCl ----- Fabric <hr/> cm ³ /gm
Bulk Volume (calipers)	---	3.32
Bulk Volume (porosimeter)	1.218	1.285
Skeletal Volume	0.773	0.641
Intrusion Volume	0.313	0.644
Interparticle (>10 μ m)	0.248	0.408
Pores (<10 μ m)	0.065	0.236
1.0 - 10 μ m	0.008	0.025
0.1 - 1.0 μ m	0.008	0.074
<0.1 μ m	0.049	0.137

Physical property information is most useful as a quality control parameter. It is also helpful as a correlating parameter with in vitro drug release or a useful in vivo property.

E. Stability of Stored Materials

The stability of the drugs in wound dressings under military field conditions needs to be addressed. Therefore preliminary experiments were performed to determine the stability of the drug (by in vitro assay) and the stability of drug release rate (also in vitro) as a function of various storage conditions. Relatively dry conditions of storage are required in order to maintain the drug within the polymer matrix. The poly-L(-)lactide is expected to be stable, but is hydrolyzed slowly in the presence of moisture. This is the basis of the biodegradation of the polymer to lactic acid in the body. As shown by the slow release of levonorgestrel from similar microcapsules (Contract No. N01-HD-0-2847) this in vitro and in vivo degradation requires several months to achieve a significant breakdown of a poly-L(-)lactide (R.S.V. approximately 1 dl/g) microcapsule wall.

Six storage conditions were chosen (Table 21) which covered a range of temperatures, with and without laboratory levels of humidity and light. Two separate containers of each drug were prepared. One container was to be opened at the end of the first contract year for assay and release testing. The other container was to be used for two later analyses. American Can Company graciously supplied sufficient retortable pouches (M-1173 61-0.93004 heat-sealable bags) for dessicant storage. Ambient light and moisture was achieved in glass vials capped with glass wool. Absence of light was achieved with black tape around the vials. Temperatures were 40°C (chemical oven), ambient (laboratory), 4°C (refrigerator). In the analysis of the data it must be remembered that different people, using slightly different techniques took the data in different years. Hence only gross changes may be significant.

The samples which have been stored are listed in Table 22. Two early samples of povidone iodine fabric and powder were stored, but were discarded after being replaced by more appropriate samples. All of the anesthetic samples have now been removed from the testing protocol.

1. Storage of Anesthetic Wound Dressings

The release of any drug from a fabric matrix was less reproducible than from powders or microcapsules, presumably because of non-reproducible wetting of the fabrics. Benzocaine fabrics may be considered to be relatively stable, although some increase in the initial release may occur after storage at 40°C (Table 23). Etidocaine-HCl in fabric form is stable. Lidocaine base is not apparently lost from the system (fabric assays are stable). However, after storage at higher temperatures, the drug is released from the fabric more rapidly.

The anesthetic release from powders (Table 24) was more reproducible, but perhaps less significant than the study of the anesthetic microcapsules (Contract DAMD17-81-C-1195). Benzocaine was lost from a 20% powder when stored, unsealed, at 40°C. There was also some redistribution

TABLE 21

SAMPLE STORAGE CONDITIONS

<u>CONDITION</u>	<u>TEMPERATURE</u>	<u>HUMIDITY</u>	<u>LIGHT</u>
1	40°C	Ambient	None
2	40°C	Desiccate	None
3	Ambient	Ambient	None
4	Ambient	Ambient	Ambient
5	4°C	Ambient	None
6	4°C	Desiccate	None

TABLE 22

SAMPLES STORED FOR STABILITY TESTSFabric Samples

<u>Drug</u>	<u>% Drug</u>	<u>Reference</u>	<u>Date Stored</u>
Benzocaine	20	8/44	7/15/81
Etidocaine-HCl	20	8/44	7/15/81
Lidocaine Base	20	8/44	7/15/81
PVP-I ₂	40	44/85	6/2/82
Benzalkonium Chloride	20		3/7/83
Ampicillin Trihydrate	20		

Powder Samples

<u>Drug</u>	<u>% Drug</u>	<u>Size (μm)</u>	<u>Reference</u>	<u>Date Stored</u>
Benzocaine	20	212-300	8/34	4/29/81
Etidocaine-HCl	20	212-300	8/34	4/29/81
Lidocaine-HCl	20	212-300	8/34	4/29/81
PVP-I ₂	20	300-425	8/44	7/15/81
Lidocaine Base	10	300-425	44/85	6/2/82
Benzalkonium Chloride	20	425-600	-	3/7/83
Nitrofurazone	40	300-425	-	--

Microcapsules Samples

<u>Drug</u>	<u>% Drug</u>	<u>Size (μm)</u>	<u>Reference</u>	<u>Date Stored</u>
PVP-I ₂	49	212-600	25/78	3/7/83

TABLE 23

STABILITY OF STORED ANESTHETIC FABRICS

(Percent of Assay Value Release at Specific Times)

Drug	Years Hours of Stored Release	Original Data	Storage Conditions					
			40°C Dark	40°C Dark	40°C Dark	R.T. Light	R.T. Dark	4°C Dark
Benzocaine 20%	1	1	8	14	14	3	1	17
		6	25	24	24	10	6	30
		24	44	37	37	23	14	45
		Assay	(20)	14	14	20	22	23
	2	1	8	24	24	3	5	12
		6	25	38	38	10	15	29
Etidocaine-HCl 20%		24	44	53	53	25	32	46
		Assay	(20)	22	22	22	22	22
	1	1	28	24	24	30	28	
		6	52	40	40	46	43	
		24	65	55	55	60	60	
		Assay	(20)	23	23	23	23	
	2	1	28	24	24	38	39	
		6	52	34	34	59	60	
		24	65	42	42	75	75	
		Assay	(20)	21	21	21	21	

TABLE 23 (continued)

STABILITY OF STORED ANESTHETIC FABRICS

(Percent of Assay Value Released at Specific Times)

<u>Drug</u>	<u>Years Hours of Stored Release</u>	<u>Original Data</u>	<u>Storage Conditions</u>					
			<u>40°C Dark</u>	<u>40°C Dark</u>	<u>R.T. Light</u>	<u>R.T. Dark</u>	<u>4°C Dark</u>	<u>4°C Dark</u>
Lidocaine Base 20%	1	36	91	Sealed	Unsealed	Unsealed	Unsealed	Sealed
	6	78	94		80	78		67
	24	96	95		80	82		72
	Assay	(20)	23		22	25		38
	2	36	103		76	89	37	71
	6	78	104		76	89	64	106
	24	96	96		78	93	78	123
	Assay	(20)	25		25	25	22	22

TABLE 24
STABILITY OF STORED ANESTHETIC POWDERS

Drug	Years Hours of Stored Release	Assay Value Released at Specific Times	Storage Conditions					
			40°C Dark Unsealed	40°C Dark Sealed	R.T. Light Unsealed	R.T. Dark Unsealed	4°C Dark Unsealed	4°C Dark Sealed
Benzocaine 20% 212-300 μ m	1	1	38	0	40	18	22	26
		6	53	1	54	34	38	38
		24	63	5	62	46	51	47
		Assay	(20)	8	20	19	17	20
	2	1	38	72			34	32
Etidocaine-HCl 20% 212-300 μ m		6	53	90			48	49
		24	63	93			60	61
		Assay	(20)	18			18	18
	1	1	44	31	40	52		46
		6	(65)	56	57	56		60
		24	75	56	60	61		66
		Assay	(20)	24	24	24		24
	2	1	44	42	51	57		
		6	-	66	74	71		
		24	75	76	74	71		
		Assay	(20)	24	22	24		

The data of USAIDR was sent to BIOTEK and was transformed into a logarithmic form and analyzed by a Student t-test. This analysis is shown in Table 31 and 32. There is a statistically significant difference between the test and control groups. However only two wounds of the test group could be considered completely aseptic, and one of these should be considered questionable since hemorrhaging occurred which required removal of the clot. Five additional test wounds had bacterial counts of less than 10^5 cfu/wash (wash is 2 ml of detergent solution). Only one control wound was less than 10^5 cfu/wash.

This data is comparable to a preliminary test (one guinea pig) in which 10 mg of pure nitrofurazone gave a value of 1.4×10^5 cfu/wash (7×10^4 cfu/ml).

In summary, 10 mg of nitrofurazone in a slow release powder reduced the standardized bacterial contamination by about 10-fold. It would be difficult to supply more than 25 mg of powder to a 1cm wound area in an effective wound dressing. However more than 40% drug might be able to be incorporated into the test powder. Faster or slower releasing powders (in in vitro tests) might be more advantageous. It is also important to show an advantage relative to the pure nitrofurazone powder (immediately available drug). Finally, a 10-fold decrease in microbial contamination is rather inadequate when compared to antibiotic treatments. Therefore, the next study was performed with ampicillin as the active material.

4) Ampicillin Studies

Ampicillin and clindamycin were tested simultaneously against S. aureus 12600. Discs of 8mm (50 mm^2) were impregnated with 10 μl of ampicillin solutions. For the trihydrate the inhibitory quantity was 0.04 μg (4 $\mu\text{g}/\text{ml}$) per 50 mm^2 . For the anhydrous ampicillin, 0.07 μg (7 $\mu\text{g}/\text{ml}$) was required. In the tube dilution test the MIC was 1.5 $\mu\text{g}/\text{ml}$ for the anhydrous ampicillin and 3 $\mu\text{g}/\text{ml}$ for the trihydrate. Sodium ampicillin was not tested because a relative assay value was available from Bristol and the wound dressings containing this material have shown less promise.

Since ampicillin was shown to be very effective against S. aureus and a promising ampicillin trihydrate fabric has been prepared, pure ampicillin trihydrate was placed in the inoculated guinea pig wound. Quantities of 20, 10, and 1 mg of drug were placed in the 1 cm wounds. The wounds were not aseptic, although the 20 and 10 mg test areas had lower colony forming unit counts. The wound areas were washed after 3 days of exposure. The control wound had 2×10^8 cfu, the 1 mg wound had 1×10^7 cfu, the 10 mg wound had 8×10^6 cfu, and the 20 mg ampicillin wound had 3.5×10^6 cfu in the wash. This compares to 80 ng/cm^2 of drug being effective on agar plates.

In the next experiment ampicillin trihydrate fabric (20%) was placed in the standardized guinea pig wound which was infected with 1.6×10^9 cfu of S. aureus 12600. Five animals of control and five test animals were sacrificed at Days 1 and 2. There was a significant decrease in the colony counts at Day 2 and it was suggested that the remaining groups be

Table 30

EFFECT OF POVIDONE IODINE PREPARATIONS
ON CONTAMINATED WOUND

Log (CFU/ml) as Mean \pm S.D. (no. of animals)

	<u>Group</u>	<u>Wound</u>	<u>Dressing</u>
1	No Bandage	6.63 \pm 0.87 (5) 6.24 \pm 0.08 (4)	No bandage No bandage
2	No drug Bandage	6.02 \pm 0.09 (4)	6.01 \pm 0.54 (4)
3	No drug Bandage + 22 mg drug	5.32 \pm 0.17 (5)	4.86 \pm 0.66 (5)
4	Drug Bandage + 22 mg Drug	5.96 \pm 0.46 (5)	6.09 \pm 0.29 (5)

Drug in dressing is approximately 8 mg (8 mg/cm²)

groups of 5 animals were used as shown in Table 30. Both the dressings and the wound areas were analysed for bacteria with a spiral plater. Scrubs were taken from the wound with 2 ml of detergent buffer, and dressings were placed in 1 ml of buffer.

The data were transformed to a logarithmic base for ease of analysis and for the appropriate student-t test. The only significant effect was the decrease of wound and bandage microorganisms when using the non-medicated bandage with pure povidone iodine (Group 2 vs. 3 for wound $t = 7.4$ and for dressing $t = 2.8$). However the counts of 2×10^5 cfu/ml of Group 3 are still "non-sterile". In these experiments the bandages were not pre-wet with saline. Thus the absorptive property of the bandage was used to our advantage, as in the preliminary experiment.

In a peritonitis model, S. aureus was used in preliminary experiments. The infection was too rampant to be controlled by antibiotics or antiseptics. Deaths occurred within hours. Using E. coli as the inoculum generated a more realistic infection (9 of 10 died in a day). This was controlled by an antibiotic preparation (0/10 died), but not with 24 mg of povidone iodine (9/10 died).

3) Nitrofurazone Studies

When a sample of nitrofurazone arrived at BIOTEK a sub-sample was sent to Dr. Vincent, along with a microbiological test method which we received from the supplier, Norwich-Eaton Pharmaceuticals. Using this method, minimum effective concentrations of nitrofurazone were determined against the two strains of S. aureus. The concentration of 6.2 ug/mg was effective against Strain 6538 and 12.5 ug/ml was required for Strain 12600. These results were based on the visible turbidity of solutions after one day at 36°C. Aliquots of each dilution were also evaluated by disc diffusion and the results of this test were inconclusive.

Two guinea pigs were used with a 1.3×10^9 cfu S. aureus 12600 inoculum after exposing subcutaneous adipose tissue using the flap technique. In one guinea pig, 10 mg of pure nitrofurazone was used. The wound sample contained 1.4×10^5 cfu/wash. In the second animal 5 mg of nitrofurazone was used and the wound contained about 1.6×10^6 cfu/wash. Thus more than 10 mg of nitrofurazone appears to be required to control this type of infection, when using this method of analysis.

In the next experiment 40% nitrofurazone powder (300-425 um), 25 mg (10 mg drug) was used in each of 15 guinea pigs. A separate group of 15 guinea pigs were used as controls (no powder or drug added). Each group of 15 was split into thirds and sacrificed at one, two, and three days after the inoculation and implantation of test material.

In the first group of animals (1 day after powder implantation) it appeared that about 50% of the nitrofurazone powder remained unchanged. This might indicate that less than the anticipated amount of drug had been released from the powder. The powder should turn from brown to white as the drug is released.

TABLE 29
 SAMPLES SENT FOR IN VIVO
 EVALUATION ON WOUND HEALING OF ARTIFICIALLY
 CONTAMINATED WOUNDS

<u>Sample</u>	<u>Fabric Wt. mg/cm²</u>	<u>Drug %</u>	<u>Drug Wt. mg/cm²</u>
Control - A	14.8	0	0
Control - B	20.9	0	0
Control W 1% SDS	21.4	0	0
Control W 0.1% SDS	30.7	0	0
PVP-I ₂	19.8	40	7.9
BAC	15.4	2.7	0.42
NITROFURAZONE	8.9	20	1.78
Ampicillin Trihydrate	17.5	20	3.50
Ampicillin Trihydrate	10.1	40	4.04
Ampicillin Trihydrate	12.6	60	7.56
Ampicillin Trihydrate	16.8	20	3.36
1% SDS			
Ampicillin Trihydrate	20.5	40	8.20
1% SDS			
Ampicillin Trihydrate	26.3	40	10.52
0.1% SDS			
Ampicillin Anhydrous Powder	---	20%	---

determined at 540 nm. In control cultures growth was log linear for at least 6 hours. In all studies of ampicillin release there was no growth. The fabric releases ampicillin at a rate which establishes the MIC (\leq 0.1 ug/ml) in 1 hour or less.

c. Antimicrobial Testing at USAIDR

The microbiological testing of the antiseptic and antibiotic wound dressings has been performed by the contracting organization (U.S. Army Institute of Dental Research) under the direction of Colonel Jack W. Vincent. Both in vitro microbial sensitivity and in vivo wound sepsis and healing tests are being performed by the Army personnel. The materials sent to USAIDR for in vivo evaluation are listed in Table 29.

Initially a test protocol was written at USAIDR (Vincent, Setterstrom, Hollinger, 2 November 1982, "In Vivo Evaluation of a Wound Dressing Containing Poly-L(-)lactide and Povidone Iodine"). This included both the effect of wound dressings on antiseptic efficacy and the effect on wound healing.

1) Preliminary Study

Strips of non-woven fabric which were at least 1.5 cm wide and about 8 cm long were sent to Dr. Vincent. The backing was Parke-Davis Gauze bandage. The strips can be cut with surgical scissors to give the correct dimensions for each wound. In order to achieve continuous release of a small quantity of BAC, a 20% BAC/polymer solution was sprayed on top of a polymer fabric. In vitro studies indicate a continued release of similar samples. The samples were handled with surgical gloves and heat sealed in plastic envelopes prior to shipment.

In this first study, ten animals were used with each type of bandage for measurement of the antiseptic efficacy. The same technique had been used to evaluate another wound dressing generating reproducibly (100% morbidity) contaminated wounds (Vincent, et al, 1982, personal communication test protocol). However, the BIOTEK fabric wound dressings were more absorbent and the control dressings provided contaminated wounds in only 3 of 10 cases (30%). In test animals, 0% of the povidone iodine dressings produced contaminated wounds (0/9) and 10% of the BAC treated sites were contaminated (1/10). Plans were made to modify the procedure to insure infection at the control sites when using polylactide control dressings.

2) Povidone Iodine Studies

In the next set of experiments both povidone iodine and nitrofurazone were studied by microbiological in vitro and in vivo methods. By using povidone iodine on agar discs a minimum povidone iodine concentration was determined which was effective against the Staphylococcus aureus strains being used in the in vivo studies. A zone of inhibition was found at 15 mg/ml for both Strain 12600 and 6538P.

A guinea pig study was then initiated with a inoculum of 1.5×10^9 S. aureus 6538P microorganisms under a full thickness dissection flap. Four

TABLE 28
B. SUBTILIS INHIBITION BY PVP·I₂ IN FABRIC

Tube	PVP·I ₂ ** mg	PVP·I ₂ (fabric) mg (PVP·I ₂)	Bag(b) None(n)	Cells*(c) Spores(s)	Absorbance of 670nm (Turbidity) 2 Hr.	4 Hr.	24 Hrs.
1	200	-	b	c	0.25	0.86	1.44
2	<u>300</u>	-	b	c	0.23	<u>0.39</u>	1.83
3	<u>400</u>	-	b	<u>c</u>	0.21	0.29	<u>0.21</u>
4	200	-	n	c	0.34	1.07	1.90
5	<u>300</u>	-	n	c	0.25	<u>0.62</u>	1.86
6	<u>400</u>	-	n	<u>c</u>	0.22	0.31	<u>0.23</u>
7	-	200	b	c	0.25	1.04	1.66
8	-	<u>300</u>	b	c	0.23	<u>0.38</u>	1.67
9	-	<u>400</u>	b	<u>c</u>	0.18	0.21	<u>0.07</u>
10	-	<u>300</u>	n	c	0.28	<u>0.45</u>	1.82
11	30	-	b	s	0.02	0.02	1.80
12	100	-	b	s	0.04	0.04	1.78
13	<u>300</u>	-	b	<u>s</u>	0.06	0.06	<u>0.05</u>
B ₁	-	-	b	-	0.02	0.02	>2.0
B ₂	-	-	n	-	0.01	0.01	0.02

* 1 ml of 24 growth cells added at 0, 1, 2 hours

** PVP·I₂ added at t=0 to 39 ml of TS Broth

release drug, controlled the bacterial growth, although there was some early bacterial growth.

At this point the technique was refined. A microcell was purchased which requires only 1 ml of solution. For analysis this solution was decolorized with 10 ul of 130 mg/ml sodium thiosulfate solution. Finally drug preparations were maintained in diffusion bags (polyester screen) to aid in solution transfers.

In the next experiments B. subtilis was added at 0, 1, and 2 hours as in Tube 8 (Table 27). Pure povidone iodine was compared with the 40% povidone iodine in fabric. In the first of these experiments all of these tubes grew bacteria (Abs > 1.7 at 24 hours for 100, 150, 200, 250, 300 mg povidone iodine as drug pure or in fabric). There was an indication at four hours that the pure drug was more inhibitory to bacterial growth than the fabric containing the drug. In the next experiment 200, 300, 400 mg of povidone iodine, as pure drug and in fabric, was measured with and without diffusion bags. Blanks were run with and without bags, without B. subtilis. The results are shown in Table 28. A ranging experiment was also performed with B. subtilis spores (Tubes 11-13).

In this experiment it is evident that 300 mg povidone iodine (per 40 ml broth) is no longer bacteriostatic to three challenges of 1 ml of the 24-growth medium; but 400 mg is bacteriostatic. With 300 mg of povidone iodine as drug or fabric, the inhibition at 4 hours is similar (0.39 and 0.62 versus 0.38 and 0.45). The blanks indicate that handling of the bags (and samples) causes insterility producing a heavy microbial growth within 24 hours. However, we believe this is not significant with respect to the challenge of B. subtilis cell inoculation.

Thus there was no proven advantage (nor disadvantage) to the use of povidone iodine fabric. The fabric is easy to apply and may cause less of a toxic or burning effect than the same amount of povidone iodine in solution. Alternately the slow release might be more effective in a test system which more closely simulates a wound condition.

b. Ampicillin Evaluation in Culture

A culture of B. subtilis was started in tryptic soy broth from the spore suspension previously discussed. The culture was plated out and a single colony was selected to start our stock culture. The culture was maintained by transfer of 100 ul aliquots to fresh media three times a week. The culture is sensitive to ampicillin. Both commercial ampicillin sensitivity disks and disks prepared from ampicillin trihydrate fabric (3 mm diameter, 24.8 ug/mm) gave large zones of inhibition.

The objective was to determine the time required for the non-woven fabric to release a minimum inhibitory concentration (MIC) of drug. The test was carried out in a modified Franz diffusion cell containing 50 ml of tryptic soy broth inoculated with approximately 70,000 c.f.u. (0.5 ml of stock culture). Either 3 or 6 mm diameter disks of fabric were placed on a filter paper disk at the top of the diffusion cell. The culture was mixed with a small magnetic stir bar and incubated at 37°C. At various times the cell was placed in a spectrophotometer and the optical density

TABLE 27
INHIBITION OF BACILLUS SUBTILIS GROWTH
BY PURE PVP·I₂

Tube No.	PVP·I ₂ mg	B. Subtilis ml	Absorbance at Various Times				
			0	1	2	4	24 (Hrs.)
1	0	0	0.01	0.01	0.01	0.01	0.01
2	0	3	0.22	0.34	0.72	1.42	3.3
3	100	1	0.10	0.11	0.15	0.73	3.3
4	100	3	0.22	0.30	0.58	1.17	2.6
5	200	3	0.22	0.23	0.22	0.22	0.12
6	300	3	0.27*	0.22	0.21	0.22	0.17
7	100	3	0.22	0.30			
	+100 (1 hr)			0.30	0.30		
	+100 (2 hr)				0.32*	0.28	0.21
8	300	1	0.20*	0.14			
		+1 (1 hr)		0.22	0.21		
		+1 (2 hr)			0.31	0.29	0.20

*distinct blue color of povidone iodine

implanted microcapsules gave variable responses. A larger pledget of fabric should be used to deliver more drug.

The testing of anesthetics by this method was successfully used on Contract DAMD17-81-C-1195, with etidocaine microcapsules producing nerve blocks which lasted for 48 hours. However, the testing of anesthetics was deleted from the work scope of this contract at this point.

3. Antiseptic/Antibiotic Testing

A wound model has been developed at USAIDR in the guinea pig and this model was used for most of the biological testing of the antiseptics and antibiotics. Some preliminary testing of the povidone iodine and ampicillin fabric was performed at BIOTEK using Bacillus subtilis culture system.

a. Povidone-Iodine Evaluation in Culture

Povidone iodine releases iodine as required to maintain an iodine concentration sufficient to oxidize amino acids of the proteins of bacteria, fungi, etc. (Schelziner, Mahinka and Levin, 1979). This available iodine will also react with local tissue protein. However, systemic metabolism of the byproducts occurs long after the oxidizing activity of povidone iodine is spent. Hence many of the antiseptic properties of the slow release povidone iodine preparations can be assessed by bacterial culture techniques, without involving a live animal.

Bacillus subtilis was used as the test microorganism. This non-pathogenic bacteria was suggested by USAIDR personnel and was used by Lowbury, et al (1964) as a test microorganism in povidone iodine studies.

B. subtilis spores were obtained from Raven Biological Laboratories, Inc., Omaha, Nebraska (ATCC No. 9372, 10 spores/ml). Tryptic soy broth from Difco (Bacto, dehydrated, pH 7.3) was chosen as the culture medium. This was prepared at 30 g/l and sterilized in the test. Cells were grown overnight in this medium and then aliquots were transferred to test cells with sterile media which contained various antiseptic preparations.

In the first experiment 100 ml of broth was used in 250 ml erlenmeyer flasks. Overnight growth from 0.1 ml of spores leveled off at about 1.3 absorbance units, at 670 nm. Ten milliliters were used to inoculate the test flasks. Various quantities of povidone iodine (BASF 17/12) were tested, and 1.0 grams of drug (1%) was found to be bacteriostatic, whereas 0.3 grams (0.3%) was not. In the second experiment 37 ml of broth was used in the standard diffusion cells. The normal inoculum was 3 ml of a 24-hour growth. The data of this experiment are shown in Table 27.

This experiment showed that 0.5% povidone iodine in this broth is bacteriostatic. This required 200 mg of povidone iodine. A concentration of 0.25% was not bacteriostatic, even with 1/3 the challenge of B. subtilis. Splitting the "standard" challenge into 3 equal increments, as in reinfesting, was controlled by a single dose of 300 mg (0.75%) of povidone iodine. Splitting the drug into three equal doses, as for a time

reagent five to seven minutes after collection on the dressing, then the correct absorbance (0.30-0.35) is obtained by the time the sample is reacted for five minutes and the fibrous dressing is centrifuged down to the bottom of the tube. The fibrous mat does not have a residual blood stain.

This method was never tested with a series of wound dressings because the use of a hemostatic agent was believed to be contraindicated in certain wound cases, and this part of the work scope was terminated.

2. Anesthetic Effectiveness

We had initially proposed to evaluate anesthetic preparations in guinea pigs by measuring the response to a tactile stimulus. However, our consultants from Astra Pharmaceuticals suggested that the change in the normal stance of a rat due to blockage of the sciatic nerve is a more reproducible method of determining local anesthetic effectiveness. Most of this work has been performed on a parallel contract (DAMD17-81-C-1195).

In order to use this approach with fabrics or large powders, it would be necessary to cut through skin and implant the material near the sciatic nerve. These materials cannot be injected through a hypodermic needle. Preliminary experiments were therefore performed to develop this method.

In the first experiment we used 45 mg/kg of ketamine plus 5 mg/kg of xylazine as the general anesthetic. Thus we used 18 mg of ketamine and 2 mg of xylazine as an intramuscular injections in 400 gram rats. We rolled a 1 cm x 1 cm piece of fabric into a 1 cm long x 0.3 cm diameter pledget. The rat thigh was shaved and scrubbed, and a 2 cm incision was made parallel to the femur. The muscle layer was dissected until the pledget could be inserted adjacent and parallel to the femur. The incision was then closed with wound clips.

Control experiments were performed in which polymer fabric (negative) and etidocaine microcapsules (positive) were implanted by a similar procedure. A sciatic nerve block could not be differentiated from general anesthesia for the first two to three hours, and no nerve block was observed with the etidocaine microcapsules or with the fabric control. Some anesthesia was observed in the rat with etidocaine fabric but this wore off within 12 hours.

In a second experiment rats were anesthetized with 40 mg/kg of sodium pentobarbital, administered intraperitoneally. In this case a 3 cm incision was made and the muscles were divided by blunt dissection. The sciatic nerve was found and gently freed from the surrounding tissue with forceps. The anesthetic preparation was inserted and the area wetted with 0.25 ml of sterile saline.

In this experiment 20 mg of etidocaine as pure drug crystals, 40 mg in microcapsules, and 7 mg in fabric (1 x 2 cm) as well as control fabric was tested. The animals recovered in 1 to 2 hours and nerve blocks were observed. The pure drug was effective for about 6 hours and the etidocaine fabric gave an anesthetic response for about one hour. There was no nerve blockage due to implanting the pure polymer fabric. The rat with

TABLE 26

STABILITY OF STORED ANTISEPTIC POWDERS

(Percent of Assay Value Released at Specific Times)

<u>Drug</u>	<u>Years Hours of Stored Release</u>	<u>Original Data</u>	<u>Storage Conditions</u>					
			<u>40°C Dark</u>	<u>40°C Dark</u>	<u>R.T. Light</u>	<u>R.T. Dark</u>	<u>4°C Dark</u>	<u>4°C Dark</u>
PVP-I ₂ 20% 300-425 µm	1	72	93	Sealed	Unsealed	Unsealed	Unsealed	Sealed
	6	79	93		76	78		
	24		93		76	78		
	Assay	(20)	15		16	17		
	2	72	80		90	97		
	6	79	80		90	90		
	24		82		95	93		
	Assay	(20)	15		15	15		

TABLE 25

STABILITY OF STORED ANTISEPTIC FABRICS

(Percent of Assay Value Released at Specific Times)

<u>Drug</u>	<u>Years Hours of Stored Release</u>	<u>Original Data</u>	<u>Storage Conditions</u>					
			<u>40°C Dark</u>	<u>40°C Dark</u>	<u>40°C Dark</u>	<u>40°C Dark</u>	<u>40°C Dark</u>	<u>40°C Dark</u>
			<u>Unsealed</u>	<u>Sealed</u>	<u>Unsealed</u>	<u>Light</u>	<u>Dark</u>	<u>Sealed</u>
PVP-I ₂ 40%	1	72	85		78		75	
	6	100	95		89		91	
	24	119	100		97		96	
	Assay	(40)	38		38		38	

of benzocaine in the sealed container at 40°C. This led to a burst of benzocaine release from these samples. Etidocaine-HCl powders were stable under all conditions. Lidocaine-HCl in 20% powder did not have significant slow release properties. The assay of the powders after storage was consistent with no decomposition of the material. Lidocaine base is a low melting compound which appeared to redistribute at all temperatures, but more rapidly at high temperature. However the total lidocaine in the powder was constant in these powders.

2. Storage of Antiseptic Wound Dressings

Fabrics and powders of povidone iodine did not demonstrate significant sustained release. However the materials were stored to assess the stability of BASF 17/12 povidone iodine under various storage conditions. The povidone iodine was stable under these test conditions (Tables 25 and 26).

F. Biological Testing

1. Hemostatic Effectiveness

The hemostatic evaluation of Avitene was studied using a uniformly bleeding wound generated by a dermatome. The method of Wilkinson, Tenery and Zuffi (1973) was modified for use on a rabbit. A Goulian skin graft knife (E. Weck Co., Research Triangle Park, N.C.) was used.

In a preliminary experiment, one rabbit was anesthetized with ketamine and a bleeding surface prepared, using a 0.012 inch blade guide. Our technique yielded a relatively uniform bleeding surface.

This surface was blotted with a sponge and then covered for five minutes with a sectional dressing of Avitene™ and cotton (U.S.P.). This test dressing was applied without a gauze covering, but was then replaced by a standard sterile dressing after five minutes. The blood on the sectional dressing was analyzed using a commercial cyanmethemoglobin reagent (Drabkin, Baker Diagnostics) and compared to the assay using rabbit blood drawn with an anticoagulant. For the single preliminary experiment approximately 16 microliters of blood was absorbed into Avitene™ and 62 microliters absorbed into cotton.

In later experiments more professional skin grafts were obtained, but even less bleeding was produced. More intense bleeding was sought by warming the area with a heat lamp, using less ketamine, and using lidocaine (vasodilator) as a topical anesthetic.

A second problem was that dried blood on Avitene™ and cotton is difficult to remove, and a stain remains after five minutes immersion in the cyanmethemoglobin reagent. Experiments were, therefore, performed with known volumes (20 microliters) of freshly drawn blood, placed directly in the reagent and after storage on Avitene™ and cotton. The reagent color is stable for at least one day after addition of blood. With dried blood, a long reaction time (hours to days) is required to obtain the correct absorbance. However, if the sample is placed in the

TABLE 24 (continued)
STABILITY OF STORED ANESTHETIC POWDERS

(Percent of Assay Value Released at Specific Times)

<u>Drug</u>	<u>Years Hours of Stored Release</u>	<u>Original Data</u>	<u>Storage Conditions</u>					
			<u>40°C Dark</u>	<u>40°C Dark Unsealed</u>	<u>R.T. Light Unsealed</u>	<u>R.T. Dark Unsealed</u>	<u>4 °C Dark Unsealed</u>	<u>4 °C Dark Sealed</u>
Lidocaine-HCl 20% 212-300 µm	1	1	70	76	82	76		72
		6	80	78	84	76		76
		24	81	80	86	78		78
		Assay	(20)	23	22	23		22
	2	1	70	91	87	82	84	86
Lidocaine Base 10% 300-425 µm		6	80	92	87	84	84	85
		24	81	99	91	92	86	87
		Assay	(20)	20	20	20	20	20
	1	1	18	94	81	82	32	56
		6	30	92	81	81	48	54
		24	41	100	90	90	66	74
		Assay	14	14	14	13	14	14

TABLE 31.

USAIDR Data On Nitrofurazone Powder
Implantation In Guinea Pigs

	<u>cfu/ml</u>		<u>log (cfu/ml)</u>	
	<u>Control</u>	<u>Test</u>	<u>Control</u>	<u>Test</u>
Day 1	4.0×10^7	0 (1)	7.60	(0)
	4.4×10^5	4.0×10^4	5.64	4.60
	3.5×10^4	1.5×10^5	4.54	4.18
	1.5×10^5	1.2×10^4	5.18	5.08
	3.9×10^5	4.5×10^4	<u>5.59</u>	<u>4.65</u>
Average	8.2×10^5	4.4×10^4	5.71	4.63 (4)
S.D.			± 1.14	± 0.37
Day 2	1.4×10^7	0 (1)	7.15	(0)
	8.6×10^6	2.8×10^6	6.93	6.45
	3.7×10^6	7.7×10^5	6.57	5.89
	3.8×10^6	2.0×10^6	6.58	6.30
	4.8×10^6	2.7×10^4	<u>6.68</u>	<u>4.43</u>
Average	7.0×10^6	1.1×10^6	6.78	5.77
S.D.			± 0.25	± 0.92 (4)
Day 3	7.8×10^6	1.8×10^5	6.89	5.25
	8.0×10^6	8.0×10^2	6.90	2.90
	5.0×10^6	3.4×10^5	6.70	5.53
	1.2×10^6	6.8×10^5	6.08	5.83
	<u>1.0×10^6</u>	<u>6.0×10^6</u>	<u>6.00</u>	<u>6.78</u>
Average	4.6×10^6	1.4×10^6	6.51	5.26
S.D.			± 0.44	± 1.43

TABLE 32

ANALYSIS OF TOTAL GROUP
(USAIDR Data)

	<u>n</u>	<u>log (cfu/ml)</u>	<u>cfu/ml</u>
Control	15	6.335 \pm 0.819	2.16x10 ⁶
Test	15	4.525 \pm 2.089	3.35x10 ⁴
	14	4.848 \pm 1.736	7.05x10 ⁴
	13	5.221 \pm 1.074	1.66x10 ⁵

$n_1 = 15$	$n_2 = 15$	$t = 3.126$	d.f. = 28	$p < .005$
$n_1 = 15$	$n_2 = 14$	$t = 2.986$	d.f. = 27	$p < .005$
$n_1 = 15$	$n_2 = 13$	$t = 3.112$	d.f. = 26	$p < .005$

sacrificed at six days. At this time four of the five wounds were essentially sterile (Table 33).

Based on this information the remaining animals of this lot were treated with the 20% ampicillin trihydrate and 1% SDS fabric. This should release ampicillin more rapidly into the area of the wound. Only two times could be chosen, which were 2 and 6 days. The data, shown also in Table 33, shows again a sterile wound at six days (5 of 5). However, again the wounds were not sterile at Day 2.

In addition fabrics with higher drug loading were also tested. The data for all of the ampicillin fabric and powder efficacy studies at the end of six days of treatment are summarized in Table 34. The most efficacious treatment was the 20% ampicillin fabric with or without S.D.S. The inclusion of S.D.S. doses give a cleaner appearing wound (J.W. Vincent, personal communication). The observation that increasing levels of ampicillin were less efficacious was surprising.

Approximate levels of ampicillin were measured at USAIDR using the microbiological test method and knowing the minimum inhibitory concentration for the microorganism. From this data (Tables 35 and 36) it appears that ampicillin is rather rapidly delivered by the test fabrics. Also there is less ampicillin remaining in the fabric containing SDS than in the fabric without SDS and a higher concentration of drug in the wound when SDS was included. In all cases there was still active ampicillin present in the dressing and the wound at the end of treatment. This is surprising since it does not agree with the efficacy study. Ampicillin was detected in guinea pig serum only occasionally on the first day of treatment.

5) Clindamycin and Chlorhexidine Diphosphanilate Studies

The Upjohn sample of clindamycin-HCl was tested at USAIDR against the S. aureus strain which was normally used for wound inoculation (ATCC 12600). After seeding Mueller-Hinton agar plates, all dilutions of clindamycin (1.0 - 0.002 mg/ml) showed a hazy area of growth, with two rings. This was indicative of some growth inhibition, but not of good antibiotic susceptibility. In tube dilution tests there was growth of the microorganism at all dilutions. Based on this information we should not proceed to test our wound dressings using this strain of S. aureus in guinea pigs. The result is surprising since clindamycin is known to be effective against 96% of the S. aureus strains.

The chlorhexidine diphosphanilate was not effective against S. aureus and a different bacteria will have to be used to test this drug.

TABLE 33

AMPICILLIN FABRIC IN GUINEA PIG WOUNDMICROBIAL COUNTSExperiment 1 - 20% Ampicillin Trihydrate Fabric (1.6×10^9 cfu, $t = 0$)

	<u>1 day</u>	<u>Mean cfu's</u> <u>2 day</u>	<u>6 day</u>
Control			
Wound	9.0×10^6	3.8×10^7	8.8×10^6
Bandage	3.0×10^6	1.3×10^8	1.6×10^7
Total	1.2×10^7 (5)	1.7×10^8 (5)	2.5×10^7 (5)
Experimental			
Wound	6.0×10^6	3.9×10^6	11
Bandage	5.6×10^6	6.5×10^5	4
Total	1.2×10^7 (5)	4.6×10^6 (3)**	15 (4)*

* One animal not sterile, 4.4×10^5 cfu wound 4.7×10^5 cfu bandage

** Two contaminated

Experiment 2 - 20% Ampicillin Trihydrate + 1% SDS Fabric (1.1×10^9 cfu)

Control			
Wound	-	3.8×10^6	1.7×10^6
Bandage	-	8.2×10^5	6.6×10^4
Total	-	4.7×10^6 (2)	1.7×10^6 (2)
Experimental			
Wound	-	1.6×10^6 (6)	23
Bandage	-	3.1×10^5 (5)**	14
Total	-	1.9×10^6 (5)	37 (5)*

* One contaminated

** One bandage almost sterile, not included

TABLE 34

SUMMARY OF AMPICILLIN EFFICACY
IN GUINEA PIG WOUND MODEL*

LOG C.F.U.**

<u>Experiment</u>	<u>Wound</u>	<u>Dressing</u>	<u>Total</u>	<u>Amount of Drug</u>
Controls (17)	6.57 \pm .04	5.88 \pm .30	6.74 \pm .13	none
20% Ampicillin Fabric (4)	1.04 \pm .65	0.63 \pm .63	1.18 \pm .92	4 mg Ampicillin Trihydrate
40% Ampicillin Fabric (1)	3.78	0	3.78	5.2 mg Ampicillin Trihydrate
60% Ampicillin Fabric (6)	4.68 \pm .36	3.51 \pm .34	4.79 \pm .44	7.56 mg Ampicillin Trihydrate
20% Ampicillin Fabric (5)	1.21 \pm .26	1.09 \pm .11	1.57 \pm .10	4 mg Ampicillin Trihydrate
1% SDS				
20% Ampicillin Powder (3)	4.12 \pm .74	---	4.12 \pm .74	1 mg Ampicillin Anhydrous
5 mg				
20% Ampicillin Powder (3)	3.55 \pm .71	---	3.55 \pm .71	2 mg Ampicillin Anhydrous
10 mg				

*Data provided by Dr. Jack W. Vincent, USAIDR

**Data are presented as mean log colony forming units (C.F.U.) \pm S.E.M. observed after 6 days of treatment. The number of animals in each group is given in parentheses.

TABLE 35
AMPICILLIN FABRIC IN GUINEA PIG WOUND
AMPICILLIN CONCENTRATIONS

Experiment 1 - 20% Ampicillin Trihydrate Fabric (approximately 3.8 mg)

	<u>Concentration of Ampicillin ($\mu\text{g/ml}$)</u>		
	<u>1 day</u>	<u>2 day</u>	<u>6 day</u>
Wound (2 ml total)	12 ± 7	3.9 ± 2.1	1.7 ± 1.1
Bandage (2 ml total)	258 ± 106	82 ± 53	66 ± 42
Serum	0.12 ± 0.11	0	0

Experiment 2 - 20% Ampicillin Trihydrate + 1% SDS Fabric

Wound	-	3.2 ± 3.2	2.1 ± 2.1
Bandage	-	22 ± 19	9.7 ± 6.8
Serum	-	0	0

TABLE 36

SUMMARY OF AMPICILLIN EFFICACY
IN GUINEA PIG WOUND MODEL*

AMPICILLIN CONCENTRATION**
(ug/ml)

<u>Experiment</u>	<u>Wound</u>	<u>Dressing</u>	<u>Serum</u>
Controls	---	---	---
20% Ampicillin Fabric (5)	1.74±.51	66.2±18.9	0+0
40% Ampicillin Fabric (1)	2.99	18.24	0
60% Ampicillin Fabric (6)	0.44±.05	17.65±11.65	0+0
20% Ampicillin Fabric (6)	2.07±.86	11.60±2.53	0+0
1% SDS			
20% Ampicillin Powder (3)	0.19±.9	---	0+0
5 mg			
20% Ampicillin Powder (3)	0.36±.04	---	0+0

*Data provided by Dr. Jack W. Vincent, USAIDR

**Data are presented as mean concentration of ampicillin + S.E.M. recovered with 2 ml washes of the wound, or 1 ml washes of dressing, or observed in serum following 6 days of treatment.

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